Environment Canada   Environnement Canada

0050393J DEST PVM   EXCH   COPY 03
TP 360 E5613
VOL   ISS 187 1
ENFOR PROJECT C

ENFOR Project C-187 (1)

Liquid Fuel Production From Hemicellulose

by

J.N. Saddler

Canada

[redacted]

Contractor's Final Report 1/
DSS Contract 41SS.KL229-1-4116

ENFOR Project C-187 (1)

Liquid Fuel Production From Hemicellulose

by

J.N. Saddler

Contractor: FORINTEK Canada Corp.
Eastern Laboratory
800 Montreal Road
Ottawa, Ontario
K1A 3Z5

Scientific Authority:

Dr. Ivan A. Veliky
Biological Sciences
National Research Council of Canada
100 Sussex Drive
Ottawa, Ontario
K1A 0R6

1/ This report has been reviewed by the Canadian Forestry Service and approved for distribution. Approval does not necessarily signify that the contents reflect the views and policies of the Canadian Forestry Service. Mention of trade names or commercial products does not constitute recommendation or endorsement for use.

March 1983

Table of Contents

Page

Abstract

General Introduction 1

1. Preparation of hemicellulose fractions and hydrolysis to their composite sugars
   - Introduction 3
   - Materials and Methods 5
   - Results 8
   - Discussion 11

2. Anaerobic production of acetone, butanol and ethanol by *Clostridia* grown on sugars found in hemicellulose hydrolysates
   - Introduction 13
   - Materials and Methods 16
   - Results 18
   - Discussion 23

3. Power solvent production of facultative anaerobes (mainly *K. pneumoniae*) grown on sugars found in hemicellulose hydrolysates
   - Summary 27
   - Introduction 29
   - Materials and Methods 30
   - Results 34
   - Discussion 51

References 57

Tables

Figures

List of Tables

Table 1. Lignin, pentosan and hexosan content of various lignocellulosic materials used as substrates.

Table 2. Enzyme activities of ethanol precipitated Novo cellobiase.

Table 3. Sugars released from different pretreated wood substrates after incubation with 1 mg/ml of ethanol precipitated Novo cellobiase.

Table 4. Specific cellulase and xylanase activities of culture filtrates from cellylolytic fungi.

Table 5. Sugars released from different pretreated wood substrates after incubation with concentrated culture supernates from *Trichoderma* E58.

Table 6: Substrate utilization and ethanol production of *C. thermohydrosulfuricum* on different media.

Table 7: Composition of various media used for growth of *C. thermosaccharolyticum*.

Table 8. Test the effect of various media on the glucose utilization and ethanol production of *C. thermosaccharolytium*.

Table 9. Composition of various media used for growth of *C. acetobutylicum*.

Table 10. Test the effect of media on glucose utilization and solvent production of *C. acetobutylicum*.

Table 11. Test the effect of inoculum age on substrate utilization and solvent production of *C. acetobutylicum*.

Table 12. Effect of inoculum size on substrate utilization and solvent production of *C. acetobutylicum*.

Table 13: Test the effect of shaking on substrate utilization and solvent production of *C. acetobutylicum*.

Table. 14. Effect of headspace gas on substrate utilization and solvent production of *C. acetobutylicum*.

Table 15: Effect of yeast extract on substrate utilization and solvent production of *C. acetobutylicum*.

Table 16. Utilization of substrate and production of solvents by *C. acetobutylicum* with time.

Table 17a. Effect of surface/volume ratio on glucose utilization and solvent production of *C. acetobutylicum*.

Table 17b. Effect of surface/volume ratio on xylose utilization and solvent production of *C. acetobutylicum*.

Table 18. Substrate utilization and solvent production of *C. acetobutylicum* grown on enzymatically hydrolyzed wood hemicellulose fractions.

Table 19: Substrate utilization and solvent production of *C. acetobutylicum* using sugars normally found in hemicellulose hydrolysates as substrates.

Table 20. The effect of various concentrations of glucose on growth and solvent production of *C. acetobutylicum*.

Table 21. The effect of various concentrations of xylose on growth and solvent production of *C. acetobutylicum*.

Table 22. The effect of various concentrations of mannose on growth and solvent production of *C. acetobutylicum*.

Table 23. The effect of various concentrations of arabinose on growth and solvent production *C. acetobutylicum*.

Table 24. The effect of various concentrations of galactose on growth and solvent production *C. acetobutylicum*.

Table 25. The effect of various concentrations of cellobiose on growth and solvent production *C. acetobutylicum*.

Table 26. Growth and solvent production of *A. hydrophilia* under anaerobic conditions.

Table 27. Growth and solvent production of *B. macerans* under anaerobic conditions.

Table 28. Solvent production by *A. hydrophilia* and *B. macerans* grown anaerobically.

Table 29. Solvent production of *A. hydrophilia* under aerobic conditions.

Table 30. Solvent production of *B. macerans* grown aerobically.

Table 31. Growth and solvent production of *Klebsiella pneumoniae* in various media.

Table 32. Solvent production by *K. pneumoniae* grown aerobically and anaerobically under shake-flask conditions.

Table 33. Solvent production by *K. pneumoniae* grown aerobically and anaerobically under non-shaking conditions.

Table 34. Solvent production by *K. pneumoniae* grown anaerobically on various glucose concentrations under shaking conditions.

Table 35. Solvent production by *K. pneumoniae* grown anaerobically on various xylose concentrations under shaking conditions.

Table 36. Solvent production by *K. pneumoniae* grown anaerobically on various glucose concentrations under non-shaking conditions.

Table 37. Solvent production by *K. pneumoniae* grown anaerobically on various xylose concentrations under non-shaking conditions.

Table 38. Solvent production by *K. pneumoniae* grown anaerobically on various glucose concentrations.

Table 39. Solvent production by *K. pneumoniae* grown anaerobically on various concentrations of xylose.

Table 40. Solvent production by *K. pneumoniae* grown anaerobically on glucose for extended periods.

Table 41. Solvent production by *K. pneumoniae* grown anaerobically on D-xylose for extended periods.

Table 42. Solvent production by *K. pneumoniae* grown anaerobically under $N_2:CO_2$ (80:20).

Table 43. Solvent production by *K. pneumoniae* grown anerobically under $H_2:CO_2$ (80:20).

Table 44. Solvent production by *K*. *pneumoniae* grown aerobically.

Table 45. Solvent production by K. pneumoniae grown anaerobically or under finite air conditions on pentoses.

Table 46. Solvent production by *K*. *pneumoniae* grown anaerobically or under finite air conditions on hexoses.

Table 47. Solvent production by *K*. *pneumoniae* grown anaerobically or under finite air conditions on cellobiose.

Table 48. Solvent production by *K*. *pneumoniae* grown on cellulosic substrates with large inoculum size (30%).

Table 49. Solvent production by *K*. *pneumoniae* grown on cellulosic substrates under finite air conditions (5% Inoculum used).

Table 50. Solvent production by *K*. *pneumoniae* grown on wood hydrolyzate.

Table 51. Effect of $CaCO_3$ on solvent production of *K*. *pneumoniae*.

Table 52. Effect of MES on solvent production of *K*. *pneumoniae*.

Table 53. Effect of media modification on solvent production of *K*. *pneumoniae*.

Table 54. Effect of initial pH on solvent production of *K*. *pneumoniae* grown under finite air conditions.

Table 55. Effect of initial pH on solvent production of *K*. *pneumoniae* grown under finite air conditions, using an anaerobic culture as inoculum.

Table 56. Effect of initial pH on solvent production of *K*. *pneumoniae* grown under finite air conditions in medium with high levels of trace elements.

Table 57. Effect of initial pH on solvent production of *K*. *pneumoniae* grown anaerobically (N_2).

Table 58. Effect of initial pH on solvent production of *K*. *pneumoniae* grown anaerobically, using an anaerobic culture as inoculum.

Table 59. Effect of initial pH on solvent production of *K*. *pneumoniae* grown anaerobically, using an anaerobic culture as inoculum.

Table 60. Solvent production of K. pneumoniae at an initial pH of 7.5.

Table 61. Effect of growth temperature on solvent production by K. pneumoniae grown anaerobically.

Table 62. Effect of growth temperature on solvent production by K. pneumoniae grown anaerobically using an anaerobic culture as inoculum.

Table 63. Effect of growth temperature on solvent production of K. pneumoniae grown on D-glucose under various conditions.

Table 64. Effect of growth temperatures on solvent production of K. pneumoniae grown on D-xylose under various conditions.

Table 65. Solvent production by K. pneumoniae grown on sugars present in wood hemicellulose.

Table 66. Solvent production by K. pneumoniae grown on uronic acids and end-products.

Table 67. Effect of initial sugar concentration on solvent production of K. pneumoniae grown on D-glucose.

Table 68. Effect of initial sugar concentration on solvent production of K. pneumoniae grown on D-xylose.

Table 69. Effect of inoculum size on solvent production of K. pneumoniae grown on various D-glucose concentrations.

Table 70. Effect of inoculum size on solvent production of K. pneumoniae grown on various D-xylose concentrations.

Table 71: Effect of sugar concentration on solvent production of K. pneumoniae upon prolonged incubation.

Table 72. Effect of a fed-batch system on solvent production by K. pneumoniae grown on D-glucose.

Table 73. Effect of a fed-batch system on solvent production by K. pneumoniae grown on D-xylose.

Table 74. Effect of nutrient supplement on solvent production of K. pneumoniae grown on a high concentration of D-glucose.

Table 75. Effect of nutrient supplement on solvent production of K. pneumoniae grown on a high concentration of D-xylose.

Table 76. Effect of end-products on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 77. Effect of end-products on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 78. Effect of ethanol added during fermentation on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 79. Effect of acetic acid added during fermentation on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 80. Effect of butanediol added during fermentation on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 81. Effect of ethanol added during fermentation on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 82. Effect of acetic acid added during fermentation on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 83. Effect of butanediol added during fermentation on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 84. Effect of end-products on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 85. Effect of end-products on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 86. Effect of ethanol on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 87. Effect of acetic acid on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 88. Effect of butanediol on solvent production of *K*. *pneumoniae* grown on D-glucose.

Table 89. Effect of ethanol on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 90. Effect of acetic acid on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 91. Effect of butanediol on solvent production of *K*. *pneumoniae* grown on D-xylose.

Table 92. Effect of various acetic acid concentrations on solvent production of *K. pneumoniae* grown on D-glucose.

Table 93. Effect of various acetic acid concentrations on solvent production of *K. pneumoniae* grown on D-xylose.

List of Figures

Figure 1. High Pressure gun for Steam Explosion (explosive decompression) of wood samples

Figure 2. Hydrolysis of increasing concentrations of Xylan by Novo cellobiase

Figure 3. Hydrolysis of increasing concentrations of Xylan by *Trichoderma* E58 culture filtrate

Figure 4. Effect of $CaCO_3$ on xylose utilization and butanol production of *C. acetobutylicum*

Figure 5. Growth and solvent production of *K. pneumoniae* grown anaerobically (N_2) in Kp medium on pentoses and cellobiose

Figure 6. Growth and solvent production of *K. pneumonia* grown anaerobically (N_2) in Kp medium on hexose sugars

Figure 7. Effect of initial pH on growth and solvent production of *K. pneumoniae* grown on D-glucose at 30°C for 3 d under (a) finite air conditions, and (b) anaerobic conditions (N_2 atmosphere)

Figure 8. Effect of initial pH on growth and solvent production of *K. pneumoniae* grown on D-xylose at 30°C for 3 d under (a) finite air conditions, and (b) anaerobic conditions (N_2 atmosphere)

Figure 9. Effect of MES concentration on solvent production of *K. pneumoniae* grown under finite air conditions, with initial pH 6.5, at 30°C for 3 d, on (a) D-glucose and (b) D-xylose

Figure 10. Effect of headspace gases on growth and 2,3 butanediol production of *K. pneumoniae* grown at 30°C, initial pH 6.5 under non-shaking conditions, with (a) D-glucose and (b) D-xylose as carbon source.

Figure 11. Effect of headspace gases on growth and 2,3 butanediol production of *K. pneumoniae* grown at 30°C, initial pH 6.5 under shaking conditions (120 rpm), with (a) D-glucose and D-xylose as carbon source.

Figure 12. Time course of the growth and solvent production of *K. pneumoniae* grown under finite air conditions with initial pH 6.5 at 30°C (120 rpm shaking) on (a) D-glucose and (b) D-xylose

Figure 13. Effect of surface area to volume (A/V) ratio on growth and solvent production of *K. pneumoniae* grown at 30°C, initial pH 6.5, 120 rpm shaking, with (a) D-glucose and (b) D-xylose as substrates

Abstract

Hemicellulose was derived from a variety of pretreated wood substrates such as water solubles from steam exploded aspen, aspen which had been solvent extracted, chlorited, treated with KOH then precipitated with ethanol:acetic acid, and commercial xylan. A variety of different fungi were screened for the ability of their culture filtrates to hydrolyse hemicellulose to its composite sugars. The sugars released were assayed by HPLC. Over 30% of most of the hemicellulose fractions at a 10% concentration could be hydrolysed to monosaccharides.

Three strains of Clostridia were screened to see which microorganism could produce highest amounts of solvents from sugars normally found in hemicellulose hydrolysates. C. acetobutylicum proved to produce highest amounts of butanol and conditions for maximum solvent production by this anaerobe were defined.

Six strains of facultative anaerobes were screened for their ability to produce "power solvents" from hemicellulose derived sugars, Klebsiella pneumoniae could efficiently utilize all the major sugars present in wood hemicellulose with 2, 3-butanediol being the major end product. The conditions for maximum diol production by K. pneumoniae grown on sugars normally found in hemicellulose hydrolysates were defined. A 4% solution of D-glucose and D-xylose was completely utilized within 2 days, with up to 1.96% and 2.2% 2, 3-butanediol produced respectively after growth on these substrates.

GENERAL INTRODUCTION

The recent global energy crisis has brought attention to the need for alternative non-petroleum based energy sources. Lignocellulosic wastes, in the form of agricultural and forest residues, is particularly appealing as a renewable energy source (Bisaria and Ghose, 1981; Flickinger, 1980; Rosenberg, 1980). Most research to date has dealt with the bioconversion of the cellulose portion of these lignocellulosic substrates to liquid fuels and chemical feedstocks. Although progress has been made in these areas, most processes currently defined still appear commercially uneconomical. Hemicellulose accounts for over one-third of the total carbohydrate content of biomass (Flickinger, 1980; Timell, 1967), and the efficient conversion of hemicellulose to liquid fuels is crucial in determining the economic feasibility of the bioconversion of lignocellulosic wastes on an industrial scale (Flickinger, 1980).

Little emphasis has been placed on the utilization of hemicellulose, with most research in this area carried out on D-xylose fermentation (Gong *et al*, 1981; Jeffries, 1981; Schneider *et al*, 1981). Although D-xylose is generally a major component of hemicellulose, other hexoses, pentoses and oligosaccharides are also obtained from chemical or enzymatic hydrolysis of the hemicellulose portion. Depending on the type of lignocellulosic substrates and extraction procedures, such sugar can account for 50% of the total carbohydrates (Timell, 1967). Efficient utilization of most or all of these sugars will therefore be important in improving the bioconversion efficiencies of various processes.

In our current program on the biological conversion of lignocellulosics to power solvents, we have studied various pretreatment and extraction procedures for wood. Chemical and enzymatic hydrolysis of the hemicellulose fractions has been compared and the major sugars shown to be a mixture of pentoses (L-arabinose and D-xylose), hexoses (D-glucose, D-galactose and D-mannose) as well as low molecular weight oligomers (such as D-cellobiose). Two groups of microorganisms were screened for their ability to utilize most or all of these sugars and convert them to liquid fuels. Three strains of *Clostridia* were assayed for the anaerobic production of power solvents, primarily butanol, from hemicellulose hydrolysates while the same substrates were used to compare the growth and solvent production of a variety of facultative anaerobes.

Each of these different stages of converting hemicellulose to power solvents i.e, extraction and identification of the hemicellulose, hydrolysis of the hemicellulose, conversion of the liberated sugars to liquid fuels, were studied in depth as they are all essential to the overall efficiency of the process.

1. PREPARATION OF HEMICELLULOSE FRACTIONS AND HYDROLYSIS TO THEIR COMPOSITE SUGARS

Introduction

Although most recent work (Jeffries, 1981; Wang et al, 1980) on the utilization of hemicellulose derived sugars has primarily concentrated on xylose, hemicellulose is a relatively complex component of lignocellulosic materials and depending on its origin can be composed of a variety of different hexoses, pentoses and uronic acids (Timell, 1967). In order that the hemicellulose component can be efficiently utilized it must first be extracted from the lignocellulosic complex then hydrolyzed to its component sugars which must all be readily utilized by fermentative microorganisms.

In this first part of the report, we have compared the composition and hydrolysis of commercial xylan (Sigma) and classically extracted hemicellulose (using solvent extraction to obtain holocellulose, etc.) with hemicellulose fractions which could realistically be obtained in an industrial process for converting lignocellulosics to liquid fuels. We have shown (Saddler et al, 1982) that aspen wood chips, pretreated by steam explosion and subsequently extracted by water, alkali, then mildly chlorited, can be used as a substrate for converting cellulose to glucose. The water soluble fractions described in this report can be obtained as by products in this process and the efficient conversion of this hexosan and pentosan mix to liquid fuels would greatly enhance the economics of this process.

In this preliminary study we have concentrated on the enzymatic hydrolysis of hemicellulose as the enzyme mix which apparently carries out this reaction most efficiently, is also the one derived from the fungi used to hydrolyse cellulose to glucose (Saddler *et al*, 1982). In future work we hope to directly compare the efficiencies of enzymatic and acid hydrolysis of different hemicellulose fractions.

Materials and Methods

Microorganisms and media: All of the fungi used were taken from the Forintek culture collection. A spore inoculum was used to initiate growth in shake flasks using Vogels medium (Montenecourt and Eveleigh, 1977). The fungi were grown in 100 ml quantities at 28°C on 2% Solka floc. Culture filtrates were obtained by filtration through a Whatman glass fibre filter after 8 days growth.

Assays: Soluble protein was determined directly, without precipitation, by the method of Lowry *et al* (1951) using bovine serum albumin (Sigma) as the standard. Total sugars were estimated colorimetrically using dinitrosalicylic acid reagent (Miller, 1959). Pentosans were assayed by the method outlined in TAPPI standard T223-05-71, acid insoluble lignin was assayed by the method outlined in TAPPI standard T222-05-75 and hexosans were measured by the anthrone (Herbert *et al*,1971) reaction. Monosaccharides were assayed by HPLC using a Varian 500, micropak NH_2-10 column, acetonitrile:H_2O 80:20 solvent system, flow rate of 1.5 ml/min and a R.I. detector.

Filter paper activity was determined by the method of Mandels *et al* (1976). Xylanase activity was determined by incubating 1 mg of an appropriately diluted enzyme solution with 10 ml of larchwood xylan (Sigma) in 1 ml of 0.05M citrate buffer (pH 4.8) at 50°C for 30 min. The reaction was terminated by the addition of 3 ml of dinitrosalicylic acid reagent. The tubes were placed in a boiling water bath for 5 min. then cooled to room temperature and the aborbance read at 575 nm. Endoglucanase and β-glucosidase activities were assayed as described previously (Saddler et al,1982).

Subtrates: a) Commercial substrates; xylan from larchwood (molecular weight 20,000) was obtained from Sigma Chemical Co.

b) SEA (solvent extracted aspen). Aspen powder (pass 20 mesh), was extracted with an ethanol-benzene mixture (1:2) using the method outlined in TAPPI standard T6 M-50. This material was dried, chlorited 3 times at 70°C with $NaClO_2$ solution at pH 4 using the method of Wise et al (1946), then extracted with 24% KOH under N_2 for 2 hrs at room temperature and the hemicellulose in solution precipitated with an ethanol: acetic acid mixture (10:1).

Aspen wood chips were steam exploded using a high pressure gun with a 250 cc capacity (Figure 1)

SEW-WS1 - (steam exploded wood, water solubles) Aspen wafers which had been exposed to saturated steam at 560 psi (250°C) for 40 sec were extracted at room temperature for 2 hr with water.

SEW-WS2 - (steam exploded wood, water solubles) Aspen wafers which had been exposed to saturated steam at 560 psi (250°C) for 15 sec were extracted at room temperature for 2 hr with water.

SEW-WS2-0.1%A Water solubles from aspen wafers soaked in 0.1% H_2SO_4 (0.2% H_2SO_4 based on wood) prior to exposure to saturated steam and water extraction.

SEW-Ws2-0.2%A Water solubles from aspen wafers soaked in 0.2% H_2SO_4 (0.4% H_2SO_4 based on wood) prior to exposure to saturated steam and water extraction.

Substrate Hydrolysis

A. Substrates were suspended at a fixed concentration in 0.05M citrate buffer, pH 4.8.

One millilitre of appropriately diluted culture filtrate was preheated to 50°C prior to adding it to 1 ml of the preheated substrate and incubated at 50°C. To terminate the incubation, the tubes were placed in a boiling water bath for approximately 5 min.

The contents of each tube were then filtered through a Reeve Angel glass fiber filter paper and the filtrate was used to assay for sugars.

B. Substrates were suspended at a concentration of 100 mg/ml in 20 ml of 0.05M citrate buffer, pH 4.8. Culture filtrates of Trichoderma E58 were concentrated to a protein concentration of 8 mg/ml on a rotary evaporator and again assayed for cellulase activity.

Four millilitres of the concentrated enzyme was preheated to 50°C prior to its addition of 20 ml of the preheated substrate and incubated for 24h at 50°C. The same procedure as in method A was followed to terminate the reaction and assay for products.

RESULTS

A variety of different methods for obtaining hemicellulose from aspen wood samples were initially attempted and the pentosan, hexosan and lignin content compared with that from commercially available xylan (Table 1). High pentosan values were obtained from the commercial xylan, however, 4% of this material was also detected as hexosans. The hemicellulose from the alcohol-benzene extracted aspen sample (SEA) had a pentosan content 6% greater than that of the commercial xylan while the pentosan content of the water solubles from the steam exploded samples were all in the range of 51-62% of the total material.

In previous studies we had noted that commercially available cellobiase (Novo) had a specific activity for xylanase which was even higher than its ß-glucosidase activity (Table 2). This enzyme preparation was diluted to a protein concentration of 1 mg/ml after first precipitating it with ethanol to remove the chemical inhibitors which were present. Increasing concentrations of xylan were incubated with this partially purified enzyme preparation and the amount of reducing sugars liberated during the time of incubation was measured (Figure 2). One hundred percent of the 1 mg/ml xylan sample was detected as reducing sugars after 60 minutes while over 50% of all of the other xylan concentrations were also hydrolysed after this time. The greatest amount of reducing sugars detected was after 6 hrs incubation with the 2% xylan substrate where 12.5 mg/ml reducing sugars were obtained. However this was equivalent to hydrolysis of only 62.5% of the initial substrate.

The same enzyme preparation derived from the ethanol precipitated Novo cellobiase was incubated with various pretreated aspen wood substrates (Table 3) and the amount of reducing sugars and monosaccharides liberated was measured. Fifty to sixty percent of each of these samples was hydrolysed to reducing sugars after 3 hrs incubation, with the commercial xylan being the most readily hydrolysed while hemicellulose fractions from the pretreated wood were all similarly hydrolysed. The monosaccharides liberated by the hydrolysis of these samples however were all equally low, falling within the range of 1.3-1.6 mg/ml and amounting to less than 15% of the reducing sugars detected.

To see if we could alleviate this problem a variety of different cellulolytic fungi were screened and their culture filtrates assayed for high specific xylanase activity (Table 4). All of the Trichoderma which were assayed exhibited high xylanase activity as well as high cellulase activity. Trichoderma E58 was used as the source of xylanase activity for subsequent work because of the high xylanase activity and its relatively high protein production. When the culture supernate of Trichoderma E58 diluted to a protein concentration of 1 mg/ml was incubated with increasing concentrations of xylan (Figure 3), a marked increase in hydrolysis was obtained compared to that obtained when the Novo cellobiase preparation was used. Approximately 75% of the 2% and 1% xylan preparations were hydrolysed to reducing sugars after 2.5 hrs incubation whereas 42% and 31% of the 5% and 10% xylan solutions, respectively, were hydrolysed to reducing sugars after this time. When an ethanol precipitated enzyme preparation from Trichoderma E58 was used to hydrolyse the pretreated wood substrates (Table 5) more than 50% of the reducing sugars detected were present as monosaccharides. Again the hemicellulose fraction obtained by solvent extraction (SEA) gave similar values to those obtained with the commercial xylan while the samples which had been slightly acidified prior to steam explosion were more easily hydrolysed to reducing sugars and monosaccharides than the non-acidified sample.

DISCUSSION

It is increasingly apparent that for the production of liquid fuels from lignocellulosics to become more economically attractive the hemicellulose as well as the cellulose components of woody substrates must be utilised (Flickinger, 1980; Wang and Fang, 1980). However, most workers who have advocated the use of sugars derived from hemicellulose have focused their attention on the utilization of xylose alone (Jeffries, 1981; Gong et al, 1981; Wang et al, 1980; Wang and Fang, 1980). It can be seen from this work and that of others (Clermont and Schwartz, 1952) that the composition of hemicelluloses depends not only on the source of the material but also on the method of extraction. Only 80% of commercial xylan could be detected as pentosans which corresponds to its reported structure (Timell, 1967)of an unbranched chain of xylopyranose residues with every fifth or sixth residue substituted at C2 with a 4-0-methyl-D glucuronic acid unit and a small number of xylopyranose residues substituted at C3 with arabofuranose.

We were primarily interested in utilizing the hemicellulose fraction obtained as water solubles after aspen wood had been pretreated by steam explosion, as this material can be obtained as a byproduct in the pretreatment of wood to obtain cellulose for ethanol production (Saddler et al, 1982). Initial results using commercial enzymes indicated that the hemicellulose fraction obtained after steam explosion could be as readily broken down to reducing sugars as the commercial xylan. Although about half of a 2% solution of the different hemicellulose fraction could be hydrolysed to reducing sugars, very few monosaccharides were obtained using the commercial enzyme preparation. An initial screening of some known highly cellulolytic fungi indicated that some of the strains which were high in cellulase activity also had a high xylanase activity. This was of interest as the process of enzymatic hydrolysis of the hemicellulose and cellulose would be greatly enhanced if this could be carried out by the one enzyme mixture. Further enzymatic hydrolysis was carried out using the culture filtrate from Trichoderma E58 as this strain not only has a high xylanase activity it also has been shown to have a high β-glucosidase activity which enhanced the conversion of cellulose to glucose (Saddler, 1982). This enzyme preparation hydrolysed greater amounts of the hemicellulose substrates to reducing sugars with approximately 60% of these reducing sugars detected as monosaccharides. It is probable that the enzymatic hydrolysis of hemicellulose is dependant on the synergistic action of several enzymes, as has been demonstrated with cellulose hydrolysis, and the amount of monsaccharides liberated will be dependant on the efficiency of this synergism as well as the activity of the individual enzymes. Preliminary studies have been initiated comparing acid and enzymatic hydrolysis of the different hemicellulose fractions. Although there was not enough time available to cover this work in any detail, it was apparent that the maximum yield of sugars that could be obtained using different concentrations of acid and length of hydrolysis rarely exceeded 80% of the original substrate. It would seem therefore that enzymatic hydrolysis of the hemicellulose fractions could be comparable to the efficiency of acidic hydrolysis once the optimum conditions have been defined. In future work we hope to directly compare the efficiency of enzymatic and chemical hydrolysis of hemicelluloses and the ability of different solvent producing microorganisms to utilize the liberated sugars.

2. ANAEROBIC PRODUCTION OF ACETONE, BUTANOL AND ETHANOL

Introduction

As part of our research program on the fermentation of lignocellulosics to liquid fuels, a variety of different hemicellulose fractions have been obtained as by-products using different pre-treatment methods. Analysis of these hemicellulose fractions have indicated that although xylose is the major sugar present, a variety of other monosaccharides are present in relatively large amounts.

The method which is commonly used, is to convert these sugars to liquid fuels using yeasts which are capable of fermenting xylose to ethanol (Schneider *et al*, 1981; Jeffries, 1981; Gong *et al* 1981). This approach does not take into account the range of sugars found in hemicellulose and narrows the liquid fuel produced to ethanol. Our approach consisted of screening 3 *Clostridia* strains to determine the best solvent producer. The highest solvent producer proved to be *C. acetobutylicum*. This microorganism was then assayed for its ability to produce solvents from sugars normally found in hemicellulose hydrolyates.

Those *Clostridia* screened were *C. thermohydrosulfuricum* and *C. thermosaccharolyticum* which both produce ethanol and *C. acetobutylicum* which produces ethanol, acetone and butanol. *C. thermohydrosulfuricum* was recently isolated (Wiegel *et al*, 1979) from thermal environments, and is reported to ferment cellobiose, hexoses and pentoses to ethanol. *C. thermohydrosulfuricum* is also reported to be able to ferment Solka floc to ethanol when grown in co-culture with *C. thermocellum* (Ng, Ben-Bassat and Zeikus, 1981). Similarly, *C. thermosaccharolyticum* can also grow in co-culture with *C. thermocellum* and produces ethanol from Solka floc (Wang *et al*, 1979a).

C. acetobutylicum has a long history in the fermentation industry. The Weizman process, as it is technically known, was first commercially used during the First World War for the production of acetone (for reviews see McCutchan and Hickey, 1954; Hastings, 1978 and Spivey, 1978). This process is still being used in South Africa where the substrate utilized is either molasses or corn. To fully appreciate the economics of this process, it must be remembered that the fermentation not only produces acetone and butanol but also hydrogen and CO_2 while the slop can be used as a feed supplement since it is high in riboflavin (McCutchen and Hickey, 1954). The major product, however, remains butanol and for this reason the economics and demand for this solvent are focal to the acetone, butanol, ethanol (ABE) process. Butanol is used mainly as a solvent, but it is also required to produce n-butylacetate (for the lacquer industry), glycol, ethers and esters, plasticizers, amine resins, butylacrylate and butyl methacrylate. It is currently being produced via three routes. Over 60% of the butanol is manufactured by catalytic hydrogenation of n-butyraldehyde which is produced by the oxonation of propylene and this is known as the oxo process. Another 20% of manufactured butanol is derived by the condensation of acetaldehyde to form aldol which is then dehydrated to crotonaldehyde and then hydrogenated to butanol. The fermentation process accounts for about 6% of butanol in production (Lowenheim and Moran, 1975).

The oxo process can produce butanol at a cost of 28.2 cents/pound to be sold at a market price of 32 cents/pound. The production cost of the ABE process is dependent on the initial substrate. The most costly substrate, molasses, can produce butanol at a production cost of $1.35/pound. When corn is used the price drops to 36.8 cents/pound. If a substrate such as waste water (sulfite liquor) could be used then the cost drops to 13.2 cents/pound, assuming that the waste is available at no cost (Volesky *et al*, 1981)

To effectively study the use of hemicellulose as a substrate for the fermentation of butanol, we have optimized the conditions necessary for maximum butanol production. In this initial study we have also compared the utilization of a variety of sugars normally found in hemicellulose hydrolysates.

Materials and Method

Culture Techniques: Cultures were routinely maintained in 10 mls of Reinforced Clostridia Media (Oxoid) in 60 ml serum bottles containing N_2:CO_2 (80:20) headspace (Miller and Wolin, 1974). *C. thermohydrosulfuricum* was a gift from Dr. J. Wiegel. *C. thermosaccharolyticum* strain 25773 and 7956 and *C. acetobutylicum* strain 824 were purchased from American Type Culture Collection. *C. acetobutylicum* was grown at 37°C whereas *C. thermohydrosulfuricum* and *C. thermosaccharolyticum* were both grown at 60°C.

Media tests were prepared with a 2% substrate and a redox indicator added. The media was boiled while flushing with the headspace gas used and dispensed anaerobically in 10 ml aliquots into 60 ml serum bottles. For tests involving surface to volume ratios the media was dispensed in various amounts in serum bottles of either 10, 60 or 160 mls. In tests using $CaCO_3$, this material was added to a 160 ml serum bottle prior to the addition of 100 mls of media. The serum bottles were autoclaved at 15 psi for 15 min. In tests with various concentrations of sugars, the substrate was added prior to sterilization.

Assays: The vials were uncapped and the culture fluid was used for turbidity readings at a wavelength of 660 nm. The pH of the fluid was also measured. To assay for sugars and solvents, the fluid was centrifuged at 6,000 rpm for 15 min. The supernatant fluid was used to assay for residual sugars and solvents produced. Total reducing sugars were analysed colourimetrically using dinitrosalicylic acid reagent (Miller, 1959). Solvents and acids produced were assayed using a Varian 4600 gas-chromatograph equipped with a Vista 401 micro-processor for peak integration. The supernatant fluid was injected onto a 6 ft x 1/8 in. chromosorb 101 column (mesh size 100-120). The column was operated with temperature programming from 160 to 190°C. The injection ports were maintained at 175°C and the flame ionization detector was set at 250°C. Helium saturated with formic acid (Ackman, 1972) was used as the carrier gas at a rate of 30 ml/min. Quantitation was done using isobutyric acid as an internal standard.

Results

The first part of the research project consisted of comparing the total solvents produced by *C. thermohydrosulfuricum*, *C. thermosaccharolyticum* and *C. acetobutylicum* when grown on glucose. Since solvent production is dependent on media composition, various media were used for each organism. *C. thermohydrosulfuricum* produced only 0.04% ethanol under the conditions described here (Table 6). *C. thermosaccharolyticum* was marginally better, producing 0.1% ethanol (Table 7 and 8). *C. acetobutylicum* showed the most promise producing 0.7% solvents (Table 9 and 10) under optimum media conditions. This is equivalent to a product yield of 35%. From this original screening program, we decided to focus our research on *C. acetobutylicum*.

The highest solvent yields produced by *C. acetobutylicum* were attained with either media 3 or 4 (Table 9). Since the use of ammonium sulfate or asparagine had very little effect on solvent profile (Table 10), all future experiments were carried out using media 3 which contained ammonium sulfate.

Further tests revealed that the solvent profile of *C. acetobutylicum* was not reproducible. When the organism was grown on glucose one of two solvent profiles would occur; the ratio of solvents to acid could either be high or low. These spurious results led to further investigations into the basic growth conditions necessary to achieve a high solvent to acid ratio. The first parameter tested was the inoculum age. It was found (Table 11) that a 2 to 3 day old culture was best for solvent production. Microscopic examination of these cultures revealed a high cell concentration with very few spores present. When different concentrations of inoculum were compared, a 5% inoculum size was found to be optimal for solvent production (Table 12). Since our solvent profiles were still not consistent (compare Table 11 and 12) other growth parameters were tested.

Table 13 shows the effect of shaking on solvent production and it was noted that shaking was slightly detrimental to a culture with a high solvent to acid ratio. When a culture had a low solvent to acid ratio the effects of shaking were more pronounced. In this situation, shaking caused up to a 63% decrease in butanol production.

Different headspace gas mixtures were also tested for their effect on final butanol levels (Table 14). Highest butanol production and sugar utilization were achieved when H_2 was used as the headspace gas. N_2 gave a product yield (g butanol/g substrate) comparable to H_2 but the substrate utilized was less. In both cases, the addition of CO_2 into the headspace gas was detrimental to both substrate utilization and butanol product yield.

Since yeast extract is an undefined component of the media known to contain about 16% carbohydrates, it was unsuitable for inclusion in media used in studying the carbohydrate utilization of C. acetobutylicum. In the hope of being able to eliminate, or reduce, the amount of yeast extract, various amounts of yeast extract were tested for their effect on growth and solvent production. Results reported in Table 15 indicate that 5 g/l yeast extract is needed for both optimum growth and butanol production of C. acetobutylicum grown on either glucose or xylose. Since yeast extract could not be eliminated we tested the media without any added substrate to see if butanol could be produced from the yeast extract. In this test no growth of the organism occurred after 2 days incubation and no butanol was produced.

All subsequent experiments were performed using the following culture conditions. Media 3 was used with a 5% inoculum from a 2 to 3 day old culture grown on Reinforced Clostridia Media. Hydrogen was used as the headspace gas and the culture was incubated under stationary conditions at 37°C.

The substrate utilization and the solvent profile of C. acetobutylicum grown on either 2% xylose or glucose was compared (Table 16). Ethanol, acetic acid and butyric acid were detected first during fermentation. Once butyric acid reached a peak level there was a switch to butanol production. The fermentation appeared to be completed within 48 hrs with very little increase in butanol obtained upon longer incubation. An unexpected result of this experiment was that the final glucose levels were high. Although the final butanol level was low, the butanol product yield was considered acceptable. Since this experiment was run in 160 ml serum bottles instead of 60 ml serum bottles, it seemed that the surface to volume ratio could be effecting the substrate utilization as well as the solvent production. In an experiment designed to test the relationship of the surface to volume ratio on substrate utilization and solve production the results indicated (Table 17a) that a high surface to volume ratio is necessary for optimal substrate utilization and subsequent butanol production when glucose is used as the substrate. In results for experiments using xylose as the substrate (Table 17b) the results are less clear as both butanol production and substrate utilization are low. There does appear to be the same trend of better xylose utilization with increasing surface to volume ratios.

Preliminary experiments using the wood hemicellulose hydrolysates (Table 18) as a substrate were not very encouraging since the final butanol levels were very low. Highest butanol yields were obtained when the water soluble fraction of steam-exploded wood was used as the substrate. The solvent production of *C. acetobutylicum* grown on a variety of sugars found in hemicellulose hydrolysates was compared (Table 19). Optimum growth was obtained when glucose was used as the substrate. The butanol production yield follows the order cellobiose > glucose > mannose > arabinose > xylose > galactose. The poor solvent yield from xylose is disappointing since xylose is the major hemicellulose sugar in hardwoods. In the hope of increasing xylose utilization, $CaCO_3$ was used as a buffer. Although $CaCO_3$ (Figure 4) did increase the xylose utilization, the butanol product yield was drastically affected. Similar results were found when glucose was used as the substrate.

The concentration of the various sugars can greatly alter the product yield (Compere and Griffiths, 1978) and for this reason various concentrations of each of the sugars were tested. The optimum concentration for growth and solvent production from glucose was 3% and the fermentation was finished after 2 days (Table 20). When xylose was used as the substrate (Table 21) maximal growth was detected at a sugar concentration of 2-3%. Maximum butanol production was detected using a 10% substrate concentration after 4 days incubation. The optimum concentration for growth and butanol production from mannose is 5% (Table 22). Again, as was found with glucose, growth and solvent production is completed within 48 hrs. Arabinose (Table 23) on the other hand, follows the same pattern as xylose with the fermentation continuing for up to 4 days. The optimum concentration of arabinose for butanol production was 10% while maximum growth was obtained using a 5% arabinose concentration. Galactose (Table 24) proved to be a poor substrate for butanol production. The highest concentration of 0.08% butanol was obtained when 10% galactose was the substrate. Preliminary results with cellobiose (Table 25) indicate that a high substrate concentration is not inhibitory to sugar utilization. These results were obtained after 48 hrs growth.

Discussion

Our original screening of the three organisms *C. thermohydrosulfuricum*, *C. thermosaccharolyticum* and *C. acetobutylicum* indicated that the highest solvent levels could be achieved by *C. acetobutylicum*. *C. thermohydrosulfuricum* and *C. thermosaccharolyticum* may have limited use in mono-culture conversion of hemicellulose sugars to ethanol. Other workers (Ng *et al*, 1981; Wang *et al*, 1979a) have studied these two organisms in co-culture with *C. thermocellum*. When cellulose is used as the substrate, an ethanol yield of 1.8 mol ethanol/mol anhydroglucose unit of cellulose is attained for both co-cultures. This suggests that these two microorganisms could degrade hemicellulose directly when used in co-culture with *C. thermocellum*.

C. acetobutylicum is historically a good solvent producer when grown on molasses or corn (Spivey, 1978; Hastings, 1978). Using the culture conditions described here, a butanol yield of 90% of theoretical could be obtained when a 2% glucose solution was used as the substrate.

A major part of the research project consisted of defining the growth parameters necessary for reproducible high solvent to acid profiles. The most important factors influencing the reproducibility of the solvent production are the age and history of the inoculum. It has been suggested (Gottschal and Morris, 1981) that sporulation and solventogenenis are linked in *C. acetobutylicum*. Classical methods for obtaining high solvent producing cultures include a heat-shock treatment of a spore suspension which presumably kills off the weaker spores and produces a better fermentation. This procedure is unsatisfactory to a microbiologist since it is difficult to imagine how cycles of heat-shocking can effect the pattern of fermentation (Gottschalk and Bahl, 1981). We have found that high solvent yields can be achieved when the inoculum used is a 2-3 day old culture maintained on Reinforced Clostridia Media (oxoid).

Another factor influencing the production of solvents is the surface to volume ratio. At high surface to volume ratios substrate utilization and solvent production are optimum. At lower surface to volume ratios, the results are variable. There does appear to be a trend towards poorer substrate utilization which results in lower amounts of butanol being produced.

Our preliminary results with the hemicellulose hydrolysates were discouraging since the level of butanol produced was very low. Although the solvent production was low, most of the substrate was utilized. This seemed to indicate that there were no inhibitory substances present in the hemicellulose hydrolysates. To be able to compare the results of the hemicellulose samples to theoretical values, substrate utilization and solvent production of C. acetobutylicum on the sugars normally found in hemicellulose hydrolysates were tested. The order of butanol product yield was cellobiose > glucose > mannose > arabinose > xylose > galactose.

Previous work indicated (Davies and Stephenson, 1941) that the fermentation pattern of C. acetobutylicum begins with acid production followed by a shift to solvent production. This shift is thought to occur as a result of the lowering of the pH during the acid phase. Although chalk is used as a buffer in most acetone-butanol-ethanol (ABE) fermentations (Hastings, 1978; Spivey, 1978), when calcium carbonate was used to buffer the C. acetobutylicum fermentation of xylose and glucose in our system, the sugar utilization increased while the product yield of butanol dropped. A similar drop in product yield of 82% and 88% for glucose and xylose utilization respectively has also been reported by other workers (Van der Lek, 1930) following the addition of 2% chalk to their media. It is not apparent what effect the addition of calcium carbonate has on the solvent producing system. It may be that as a drop in pH is required for the shift from acid to solvent production to take effect, the calcium carbonate may buffer too well and the sudden drop in pH does not occur even though the final pH is below the level required for solvent production. Due to the nature of the buffering action of calcium carbonate, increasing amounts of CO_2 are released into the system such that substantial amounts of CO_2 are still present in the media when fermentation is completed.

Our results using different headspace gas mixtures have shown that the presence of CO_2 is detrimental to substrate utilization and solvent production. Further studies will be initiated into the use of alternate buffers such as the organic buffer MES. In a continuous system or in larger culture volumes, the pH can be controlled with the continuous addition of base, however conditions in small vials have to be standardized since large volumes are unsuitable for the testing of various hemicellulose fractions which are only produced in small quantities.

One reason for the low production of butanol from arabinose, xylose and galactose may be due to the relatively low (2%) substrate concentration. Other workers (Compere and Griffiths, 1978) have shown that widely varying product yields were obtained when different substrate concentrations of xylose and arabinose were used. Highest butanol values were also obtained in this system when cellobiose was used as the substrate. The efficient utilization of this disaccharide could be important as cellobiose has been shown to be one of the major products resulting from enzymatic hydrolysis of most wood holocellulose fractions.

Our results indicated that glucose, mannose and galactose require a fermentation time of 2 days on any concentrations of sugar. Xylose and arabinose however require longer fermentation times when higher concentration of sugars are used. Cellobiose produces the highest butanol level achieved and the fermentation is not inhibited by high sugar concentrations. The results suggest that for optimum utilization of hemicellulose sugars, a high (5%) concentration of these sugars should be used in the media. Since fermentation time seemed to be slower on pentoses, an adaptation program might be useful for the efficient conversion of hemicellulose hydrolysates.

The fact that mannose, one of the major sugars present in softwood hemicelluloses, shows a higher product yield than xylose may indicate that hydrolysates from hemicelluloses of softwoods will be the substrates of choice for future acetone-butanol fermentation work process.

3. POWER SOLVENT PRODUCTION OF FACULTATIVE ANAEROBES (MAINLY KLEBSIELLA PNEUMONIAE) GROWN ON SUGARS FOUND IN HEMICELLULOSE HYDROLYSATES

Summary

The utilization of wood hemicellulose-derived pentoses and hexoses by microorganisms and their subsequent conversion to liquid fuels and chemical feedstocks were investigated. Six strains of facultative anaerobes were screened for their capabilities to produce power solvents from sugars. Although Bacillus macerans and Aeromonas hydrophilia strains were shown to achieve 2, 3 butanediol yields of 0.4 mole per mole of D-glucose fermented, they were incapable of pentose bioconversion. Klebsiella pneumoniae (formerly Aerobacter aerogenes) efficiently utilized all major sugars present in wood hemicellulose with the major end product being 2, 3 butanediol under anaerobic conditions. Solvent production from D-xylose was comparatively low under anaerobiosis (with N_2, N_2/CO_2 or H_2/CO_2 in headspace), but could be drastically improved by growing cells under finite air conditions. In this chemically defined medium diol yields of 0.69 mole per mole of D-glucose utilized could be obtained anaerobically, while 0.45 mole of diol per mole of D-xylose consumed was achievable under finite air conditions. Bioconversion of wood hydrolyzates was also feasible, with butanediol accumulation of 0.2 - 0.35 g/g sugars utilized.

Optimization of the fermentation process was then studied. Incorporation of 2[N-morpholino]-ethanesulfonic acid (MES) in the medium

significantly improved production of butanediol. Maximal diol yield was obtained from cultures with an initial pH of 6.5 and incubated at 30°C, although growth was optimal at an initial pH of 7.5 and temperature of 37°C. Growth and solvent production under finite air conditions was shown to surpass anaerobic cultures under all head-space gases tested (H_2, H_2/CO_2, N_2, N_2/CO_2). All the major sugars present in wood hemicellulose, at 2% final concentrations, were completely utilized in 2 days, with diol yields of up to 0.8 mole per mole of D-glucose consumed or 0.6 mole per mole of D-xylose. Efficiencies of bioconversion, however, decreased with higher initial sugar concentrations and this was only partially alleviated by supplementing the medium with various additional nitrogen sources. Studies on end-products revealed that ethanol and diol, at concentrations above the normal levels achieved from fermentation, were not inhibitory to cell growth or butanediol accumulation. Presence of acetic acid above 1.0% led to a marked inhibition of fermentation, although diol production was enhanced upon addition of 0.3 - 0.5% acetic acid, resulting in complete utilization of 4% D-glucose or D-xylose within 2 days, with up to 1.96% and 2.20% of diol being produced, respectively.

Introduction

Initially a program was established to screen for microorganisms capable of utilizing most of the sugars present in hemicellulose hydrolysates and converting these sugars to possible liquid fuels. *Aeromonas hydrophilia* and *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*) were selected for their reported capabilities to produce 2, 3-butanediol and ethanol, whereas *Bacillus macerans* was studied for its potential in acetone-ethanol fermentation.

Most of the emphasis, however, has been placed on butanediol fermentation by *K. pneumoniae*, an organism well studied in the past for its nutritional versatility and ability to produce butanediol, a valuable fuel additive. Although extensive research on the fermentation process has been carried out in the past, the lack of more recent information using present day technology prompted us to carry out more basic research on the organism, particularly with respect to optimum conditions for butanediol production. During this work, we have investigated in reasonable depth the suitability of medium used and the parameters affecting butanediol accumulation during the fermentation process.

Materials and Methods

Microorganisms: *Bacillus macerans* NRCC (National Research Council Canada) strains 9073, 9074 (ATCC 843) and 9075 (ATCC 8244), *Aeromonas hydrophilia* NRCC 1001 (ATCC 19570) and 1002, *Klebsiella pneumoniae* (*Aerobacter aerogenes* NRRL B-199), NRCC 3006 (ATCC 8724) were all obtained from National Research Council of Canada Culture Collection. Cultures were maintained on nutrient agar slants at -20°C.

Media: Media used in this study were either complex media (Bacto nutrient agar and nutrient broth, NB, Difco Laboratories, Detroit, Michigan) with or without addition of 1% D-glucose or D-xylose, or modified minimal media known to support growth of each type of organisms. Medium for *A. hydrophilia* was modified from Stanier and Adams (1944), containing (grams per litre in parenthesis), yeast extract (Difco) (6.0); calcium carbonate (4.0); $MgSO_4$ (0.1); K_2HPO_4 (0.45) and carbon source (10), pH 7.4. Medium for *B. macerans* was modified from Northrop *et al* (1919), containing 0.5% peptone (Difco), 2.0% calcium carbonate and 1.0% sugar.

Medium used for culturing *K. pneumonae* was a chemically defined medium (Kp) modified from Anderson and Wood (1962), containing (milligrams per litre in parenthesis), $K_2HPO_4.3H_2O$ (11400); KH_2PO_4 (1500); $(NH_4)_2SO_4$ (3000); EDTA (ethylenediaminetetraacetic acid) (400); $MgSO_4.7H_2O$ (240); NaCl (100); $CaCl_2.2H_2O$ (14); $FeSO_4.7H_2O$ (10); $MnSO_4.4H_2$ (2.8) and $ZnSO_4.7H_2O$ (7.5) at initial pH of 7.2. In later experiments, MES (2[N-morpholino]-ethanesulfonic acid, pKa 6.1) was added to medium routinely at 1.0% final concentration for an initial pH of 6.5 without adjustment. For pH optimum studies, initial medium pH was adjusted to 5.5, 6.0, 7.0 and 7.5 with either 5N NaOH or concentrated HCl. Calcium carbonate ($CaCO_3$), when used, was autoclaved separately in powder form and the pre-sterilized medium was added to it prior to inoculation. All sugars were prepared anaerobically (nitrogen atmosphere) in 20% or 40% solutions and sterilized either by autoclaving (121°C, 15 min) or by passage through a 0.22 mm filter (Millipore Ltd., Mississauga, Ontario). Aliquots of the sugar solutions were transferred anaerobically to pre-sterilized medium for appropriate final concentrations. For studies using high sugar concentrations, medium was prepared in double strength and then appropriately diluted with sterile distilled water and sugar solutions.

Media for aerobic cultures were prepared as 10 ml aliquots in 50 ml Erlenmeyer flasks and covered with sponge stoppers. Media for cultures to be grown under finite air supply were prepared in Wheaton serum bottles (10 ml in 60 ml vials or 50 ml in 160 ml vials) and sealed with butyl rubber stoppers and aluminum caps. Media for anaerobic cultures were prepared by the Hungate technique (1950) as modified by Miller and Wolin (1974), using O_2-free gases (Linde Specialty Gases, Union Carbide Canada Ltd., Toronto, Ontario) of N_2 or H_2, or gas mixtures of N_2/CO_2 (80:20, v/v) or H_2/CO_2 (80:20, v/v) in head space. Studies on growth cycle of the organism in different sugars were carried out in 50 ml broths in 125 ml Erlenmeyer flasks specifically adapted with side-arm tubes and modified to accept serum stoppers and caps. Studies on effect of surface area to volume ratio on solvent production were carried out by dispensing 10 ml, 25 ml, 50 ml and 100 ml medium in 160 ml serum vials, giving A/V ratio of 1.963, 0.785, 0.393, 0.196 or a medium volume to headspace gas volume ratio of 0.0625, 0.146, 0.292 and 0.625, respectively.

Wood hydrolyzates used for studies with K. pneumoniae were prepared as described in the previous section.

Culture Conditions: Working culture at -20°C was thawed at 37°C for 1/2 hr and streaked onto nutrient agar plates to be incubated aerobically at 37°C for 24 hr. Colonies were checked for purity and then used to inoculate medium broths (with 1% D-glucose). These cultures were incubated aerobically at 37°C with shaking (150 rpm) for 24 hr, then used to inoculate fresh test media (routinely with 2% inoculum, v/v) which were incubated at 30°C or 37°C (120 rpm shaking) for specified time periods. Alternatively, aerobic broth cultures were used to inoculate anaerobic media (N_2 atmosphere) for 24 hr incubation at 37°C (120 rpm shaking). The resulting cultures were then used to inoculate fresh media under various conditions. Cultures grown in 10 ml volumes in 60 ml vials were sampled whole, while those grown in 50 ml volumes in 125 ml flasks or 160 ml vials were sampled by withdrawing 2 ml asceptically at specified times under anaerobic conditions and analyzed.

Analytical Methods: Growth of the organism was monitored by reading optical density at different wavelengths in a 1 cm light path curvette using a Spectronic 710 Spectrophotometer (Bausch and Lombe, Rochester, N.Y.) Cells were harvested by centrifuging (4°C) at 10,000 x g for 15 minutes and the supernatant fluids were analyzed for volatile fermentation products by gas chromatography (Ackman, 1972), using a Varian 4600 Gas

Chromatograph (Varian Assoc. Inc., Palo Alto, California) equipped with an autosampler and Varian CDS 401 Data System. A 6 ft x 1/8 in chromosorb 101 column (mesh 100-120) (Chromatographic Specialties Ltd., Brockville, Ontario) was used with a flame ionization detector. The injection temperature and detector temperature were set at 200°C and 250°C, respectively. The column oven was operated isothermally at 190°C. Helium was used as carrier gas and the flow rate was set at 30 ml/min. Samples were quantitated with internal standard of either isobutyric acid or propionic acid.

Reducing sugars were analyzed with dinitrosalicylic acid (Miller, 1959) using the corresponding sugars as standards. Monosaccharides were assayed by HPLC (high performance liquid chromatography) using a Varian 500, micropak NH_2-10 column, acetonitrile:H_2O (80:20) solvent system, flow rate of 1.5 ml/min and a Refractive Index (R.I.) detector.

Chemicals: All salts were reagent grade (Fisher Chem. Co., Fairlawn, N.J.). D(+) cellobiose, MES, D(+) xylose, carboxymethylcellulose, glucuronic acids, galacturonic acids, salicin, xylan and propionic acid were all purchased frim Sigma Chemicals (St. Louis Missouri). 2, 3-butanediol was obtained from Aldrich Chem. Co. Inc. (Milwaukee, Wisconsin). 3, 5-dinitrosalicylic acid was purchased from Eastman Kodak Co. (Rochester, N.Y.). Cellulose powder CF11 was obtained from Whatman Ltd. (Maidstone, Kent). Avicel was purchased from FMC Corp. (Philadelphia, PA.).

Results

I. INITIAL SCREENING OF MICROORGANISMS FOR POWER SOLVENT PRODUCTION FROM HEMICELLULOSE SUGARS.

Six strains of facultatively anaerobic bacteria were studied for their abilities to utilize D-glucose and D-xylose and convert such sugars to power solvents, including acetone, ethanol, butanediol, etc. Under anaerobic conditions, *Aeromonas hydrophilia* was shown to be capable of bioconversion of D-glucose to liquid fuels (Table 26), although strain NRCC 1002 was not able to carry out such bioconversion in a minimal medium. Little growth and solvent production could be detected in nutrient broth or minimal medium, with or without 1% D-xylose supplement. Similar situations were found with three strains of *Bacillus macerans* (Table 27). However, in this case, only strain NRCC 9074 was capable of growth and solvent production on D-glucose. Solvent production of all five strains tested were low, with the highest diol yield being only 0.4 mole per mole of D-glucose utilized. Prolonged incubation of up to 30 days also failed to result in more solvent production from D-xylose (Table 28).

We then investigated growth and solvent production of the bacteria under aerobic conditions. Similarly, only low levels of butanediol was detected in cultures of *A. hydrophilia* grown on D-glucose while only trace amount of acetic acid was found with strain NRCC 1001 grown on D-xylose (Table 29). No butanediol formation was detected in cultures of *B. macerans* (Table 30), although low levels of acetic acid were present in some cultures.

Growth of K. pneumoniae in nutrient broth under aerobic and anaerobic conditions (N_2 atmosphere) did not result in significant solvent production (Table 31). Addition of D-glucose or D-xylose as a supplementary carbon source resulted in accumulation of ethanol, acetic acid and butanediol. When the organism was grown anaerobically in a chemically defined medium (Kp), diol yield of up to 0.67 mole per mole of D-glucose consumed was achieved, with complete utilization of 1.0% D-glucose. Diol yield from xylose, however, was significantly lower.

II. STUDIES ON 2, 3-BUTANEDIOL FERMENTATION BY KLEBSIELLA PNEUMONIAE.

Since K. pneumoniae showed considerably more potential in bioconversion of sugars to power solvents than B. macerans and A. hydrophilia, a more detailed investigation on the fermentation process was initiated.

All the major sugars commonly found in wood hemicellulose hydrolyzate were capable of supporting good growth of the organism under aerobic conditions (Table 32). Complete sugar utilization of a 1% solution was generally achieved in 24 hr, but little or no solvent was produced. Under anaerobic conditions (N_2 atmosphere), growth and sugar utilization were generally slower than the corresponding aerobic culture, resulting in a substantially lower biomass yield. However, both ethanol and diol production was greatly increased with anaerobiosis.

When anaerobic cultures were incubated under non-shaking conditions, growth and sugar utilization were slower and at lower levels than corresponding anaerobic cultures grown under shake-flask conditions (Table 33 vs TAble 32). Concomitantly solvent yields, particularly diol yields, of non-shaking cultures were considerably lower as well. While sugar utilization by the organism grown aerobically under standing conditions still reached completion, solvent yields in general were low (Table 33), though slightly improved over the corresponding aerobic shaking cultures (Table 32).

Attempts were then made to grow K. pneumoniae on various glucose concentrations (Table 34). Under shaking conditions, anaerobic production of ethanol and butanediol was maximal with high sugar concentration, but efficiency in sugar utilization and bioconversion to solvents decreased drastically with increases in glucose levels. Patterns of growth, sugar utilization and solvent production when D-xylose was used as the substrate under the same growth conditions were essentially identical to those of glucose , though at lower levels (Table 35). For cultures grown anaerobically without shaking, efficiency of sugar consumption also decreased with higher glucose or xylose concentrations (Tables 36 and 37). Solvent production continued to be low, with poor bioconversion efficiencies. Optimum butanediol production was shown to occur at 3% sugar concentration, while low diol yields were obtained at high sugar levels (8-10%).

A more refined study was then carried out to examine sugar utilization and solvent production at low sugar concentrations (Table 38). Complete consumption of 0.1-0.5% glucose occurred within 24 hr, however, butanediol was not detected in cultures grown on glucose below 0.5% level. With increase in sugar concentration, ethanol and diol production became maximal at 2-3% sugar concentration , but declined rapidly at higher sugar levels. Although sugar consumption was still active, (around 20 g/L glucose utilized in a 10% solution) the low culture pH probably prevented the production of more butanediol.

When D-xylose was used as the substrate, no apparent optimum for solvent production was observed (Table 39). Both growth and diol yields were poor, even though a significant portion of the xylose present had been utilized (22% consumption of a 10.0% solution).

Upon prolonged incubation on glucose or xylose, no improvement in solvent yield was detected (Tables 40 and 41). On the contrary, butanediol levels actually declined with time of incubation after 2 days, probably due to utilization of butanediol by the organism.

Since little solvent accumulation occurred when cells were grown anaerobically (N_2 atmosphere) on xylose, a study was initiated to investigate solvent production under anaerobic conditions using other gases in the headspace. A slight improvement was observed when cells were grown on xylose under N_2:CO_2 (80:20) (Table 42) or H_2:CO_2 (Table 43), but solvent yields from most other sugars decreased substantially as compared to yields detected previously in N_2-grown cells (Table 32).

Growth and solvent production of K. pneumoniae under nitrogen atmosphere were then monitored during the growth cycle in order to better understand the processes involved (Figures 5 and 6). With the exception of D-xylose grown cells which displayed a relatively long lag period (8 hr) (Figure 5) growth on all sugars tested had a short lag period before entering an exponential phase of growth and reached stationary phase in 7-8 hours. In general, ethanol production appeared to occur throughout the exponential phase and continued into stationary phase. Production of

2, 3-butanediol, on the other hand, was significant only at late logarithmic growth phase and levelled off slowly in stationary phase. At the same time, culture pH declined rapidly to below 6.0. With the notable exception of D-xylose and D-cellobiose, sugar consumption was completed within 24 hours. Growth and solvent accumulation also reached a plateau by 24 hours, with less than 10% additional accumulation of solvent resulting from 5 days incubation.

Since cells grown anaerobically on D-xylose had a long lag period which was not found under aerobic conditions, attempts were made to improve growth and solvent yield of the organism by allowing a small amount of air to be present in the culture environment at the early stage of fermentation. Preliminary experiments were set up to compare aerobic growth of K. pneumoniae under shaking and non-shaking conditions (Table 44). As noted earlier, little solvent accumulation occurred in aerobic cultures under shake-flask conditions. However, when cells were grown as standing cultures, significant solvent formation was detected early in the fermentation process. When D-xylose was used as the substrate, the butanediol yields were about three fold higher than the best diol yields previously obtained from anaerobic cultures. With extended incubation time, solvent accumulation was no longer detectable, presumably due to the diffusion of air into the system which favoured aerobic respiration of the organism over fermentative mode of energy metabolism, as one would expect.

We then proceeded to further study the role of air at initial stages of fermentation on subsequent growth and solvent production of K. pneumoniae on D-xylose as well as the other sugars. By growing the cells under finite air conditions, complete utilization of D-xylose was achieved in 1 day together with a dramatic increase in butanediol production (Table 45). When L-arabinose, D-glucose, D-galactose or D-mannose were used as the carbon source, similar growth conditions led to a slight decline in solvent yields (Tables 45 and 46). When D-cellobiose was used as the substrate, substantial improvement in diol yield was also noted, though not to the same extent as shown by the D-xylose grown cells (Table 47).

We then attempted to grow the cells on cellulosic substrates, salicin and D-arabinose (Table 48). Growth was apparent when soluble substrates were used, but butanediol production was negligible. When a heavier inoculum was used improvements in growth and solvent production were observed. Only cultures grown on xylan showed detectable levels of butanediol (Table 49), possibly due to other contaminating sugars present in the commercial xylan preparation.

The inability of K. pneumoniae to utilize lignocellulosics directly pointed to the necessity of pre-treatment and hydrolysis of wood substrates prior to fermentation. When such wood hydrolyzates were used as substrates, efficient utilization of hemicellulose-derived monosaccharides occurred within 2 days (Table 50) with high butanediol yield obtained from the enzymatically treated xylan and the solvent extracted wood. Comparatively low diol values were detected when the water soluble fraction of steam exploded wood was used as the substrate, with most of the solvents produced being acetic acid.

III. MEDIA OPTIMIZATION IN 2, 3-BUTANEDIOL FERMENTATION BY K. PNEUMONIAE.

A systematic study was carried out in order to optimize butanediol production from sugars present in wood hemicellulose, particularly with respect to D-xylose. Since the Kp medium only contained phosphate buffer, we attempted to provide extra buffering capacity at around pH 6.0 by addition of calcium carbonate at various concentrations. As observed in Table 51, $CaCO_3$ addition resulted in better growth and pH buffering, but was unsuccessful in increasing butanediol production. We then used an organic buffer MES, with pKa at 6.1. By incorporating 1.0% MES in Kp medium (with an unadjusted pH of 6.5), diol yields were greatly improved, especially when D-xylose was used as the sole carbon and energy source (Table 52).

Preliminary work on media modification was then carried out (Table 53). The deletion of EDTA in Kp medium containing D-glucose as the substrate led to improved growth, but butanediol yields dropped by almost 40%. Such an effect was only partially offset by replacing EDTA with another metal chelating agent, citric acid. By reducing the phosphate content of the culture medium, butanediol produced from glucose was improved marginally, but replacement of EDTA by citric acid in this low-phosphate medium again caused a sharp decline in diol yield. When D-xylose was used as the substrate, all modifications attempted led to a decrease in diol accumulation.

We therefore set out to pursue optimization of butanediol production in an unmodified Kp medium, except for the inclusion of 1.0% MES. Effect of the initial pH of the culture medium on growth, sugar utilization and solvent production was investigated (Tables 54-60). Under finite air conditions, maximal butanediol formation took place at pH 6.5, irrespective of carbon source (Table 54). Sugar utilization and solvent production by cultures with an initial pH of 5.5 were comparatively poorer than those at higher initial pH's. Similar results were obtained when cultures were inoculated with an anaerobic culture instead of the routine aerobic starting culture (Table 55). Optimum initial pH was still 6.5 with respect to butanediol accumulation and sugar consumption. Growth of the cells, however, appeared to be favoured with higher initial pH values.

By increasing the trace-elements concentration in the culture medium, improvement in butanediol yield was obtained (Table 56). The optimum pH remained at 6.5, although the pattern was not as clear-cut in cultures grown in D-xylose.

When cells were grown under anaerobic conditions (N_2 atmosphere) with D-glucose as the substrate, maximal diol yields were shown to occur at an initial pH of 7.0 (Table 57), corresponding to the pattern of cell growth. With D-xylose as the sole carbon source, no apparent pH optima could be noted after 1 day of incubation since diol yields were insignificant. The same phenomenom was observed when anaerobic cultures were inoculated with starting cultures grown under N_2 (Table 58), except that lower values for growth and solvent yields were achieved. With prolonged incubation the patterns of maximal diol yields was clarified (Table 59). When D-glucose was used as the substrate, growth, sugar utilization and diol production all increased steadily with an increase in the initial pH, with the pH optimal occuring at pH 7.0. When D-xylose was used as the substrate, on the other hand, optimum pH for diol yield was at 6.5, identical to cultures grown under finite air conditions. Growth and sugar consumption, however, were all favoured at a higher pH.

We then tested growth and solvent production of K. pneumoniae at initial PH of 7.5 (Table 60). By comparing results obtained with Tables 54 and 59, the experiment reaffirmed that the optimal pH for diol production was 6.5, except for cells grown anaerobically on D-glucose in which case maximal diol production occurred at pH 7.0.

Attempts were then made to optimize growth temperature for solvent production. Preliminary experiments were carried out with anaerobic cultures grown at three different temperatures. No apparent difference in diol yield was observed after 1 day of incubation, regardless of carbon source (Table 61). Upon prolonged incubation butanediol accumulation at lower temperatures was shown to surpass those values obtained at 37°C (Table 62). A more refined study was then carried out, taking into consideration the possible synergistic effects of temperature with pH, substrate and head space gases on the fermentation process. Under all combinations of such parameters tested, diol yields obtained at 30°C either equalled or exceeded the corresponding values at 37°C for cells grown on D-glucose (Table 63) or D-xylose (Table 64). In general, cells grown under finite air conditions favoured 37°C for biomass yield, though the reverse pattern was observed in anaerobic cultures. The effect of temperature on sugar utilization and ethanol and acetic acid production was not as well defined.

Once the optimum temperature for butanediol accumulation was established at 30°C, we next examined the effect of various concentrations of MES on diol yield (Figure 7). While growth and sugar utilization appeared to be favoured by an increase in MES concentration, diol yields were shown to be optimal at 1% with an initial pH of 6.5.

To ensure that the pH optima had not been altered due to growth at lower temperature (30°C), the effect of initial pH on diol yield was re-examined at 30°C (Figures 8 and 9). Under all conditions tested, growth of the organism was favoured at a higher pH, with maximum growth at pH 7.5. Accumulation of 2, 3-butanediol, however was optimal at 6.5, except for cells grown on D-glucose under anaerobic conditions, as one would expect from previous studies at 37°C.

Since 2, 3-butanediol fermentation is theoretically an anaerobic process, it is interesting to note that diol production was significantly higher when the cells were grown under finite air conditions than corresponding cultures with nitrogen in headspace (Figures 8 and 9). Studies were therefore carried out to investigate the effect of anaerobiosis, using other headspace gases, on solvent production. When the organism was grown under non-shaking conditions, the effect of headspace gases on growth and diol yield was negligible when D-glucose was used as the substrate (Figure 10a). When cells were grown on D-xylose, presence of pure N_2 or H_2 in the headspace resulted in inhibition of growth, sugar utilization and diol formation (Figure 10b). Such inhibitory effects could be eliminated by the presence of CO_2 (20%, v/v) in the headspace, restoring growth and diol yields close to those obtained in cultures grown under finite air conditions.

The effect of headspace gases became apparent in cultures grown under shaking conditions. When D-glucose was used as the substrate, growth, sugar utilization and diol accumulation of cells grown anaerobically (regardless of headspace gases) were essentially identical and of much lower values than those of cultures grown under a finite air supply (Figure 11a). A similar situation occurred when cells were grown on D-xylose (Figure 11b). Although the initial growth, sugar consumption and butanediol production were slower in anaerobic cultures without CO_2 in the headspace, no significant difference existed after prolonged incubation (Figure 11b).

The time course of the fermentation process was then studied (Figure 12). Under finite air conditions, growth, sugar utilization and medium pH decline generally proceeded with no appreciable lag period. When D-glucose was used as the substrate, growth and carbon uptake were completed within 30 hr. concurrent with maximum diol and ethanol yields (Figure 12a), whereas acetic acid production remained low even under prolonged incubation. Growth and solvent formation were slower with xylose-grown cells (Figure 12b), with maximal diol production taking place in 54 hr. The presence of 1.0% MES was shown to be effective as a buffer, successfully maintaining culture pH at around 5.5 towards the end of the fermentation.

Since studies were carried out in 10 ml or 50 ml liquid volumes in 60 ml or 160 ml vials, respectively, attempts were made to examine the effect of alterations in physical dimension of the culture vessel, particularly with respect to surface area to volume (A/V) ratio, on growth and solvent production. With D-glucose as substrate, optimal A/V ratio for diol production was demonstrated to be at 0.39 (Figure 13a). The ratio shifted to 0.79 when cells were grown on D-xylose (Figure 13b). In both cases, growth of the organism was maximal under conditions of a high A/V ratio, although sugar utilization was maximal at an A/V ratio of 0.79.

Under these established optimum conditions, solvent production from *K. pneumoniae* grown on all major sugars known to be present in wood hemicellulose were studied (Table 65). All sugars tested were found to be efficiently utilized by the organism, with 2% sugar consumed within 2 days. Diol yields from pentoses surpassed those obtained from all other sugars tested, except for D-glucose. However, diol yields from hexoses and cellobiose were generally better than pentoses on a molar basis.

When uronic acids were used as the substrates, good growth was also obtained, but diol yields were low, and the major product was acetic acid (Table 66). Growth was also obtained when lactic acid and acetic acid were used as the sole carbon source, however, but solvent production was minimal.

IV. UTILIZATION OF HIGH SUGAR CONCENTRATIONS BY *K. PNEUMONIAE* FOR BUTANEDIOL PRODUCTION

The effect of initial sugar concentration on solvent production of *K. pneumoniae* was then investigated so as to determine the capability of the organism to carry out bioconversion at high sugar levels. Preliminary results indicated that maximal diol production was obtained at a 5% sugar concentrations of either glucose (Table 67) or xylose (Table 68). However, most efficient bioconversion of sugar to solvent occurred at 1-2% sugar concentration. Growth and diol production of the organism were slower at high sugar levels (8-10%) but eventually reached the same level as cultures grown at lower sugar concentrations.

In an attempt to attain more efficient utilization of sugars and conversion to butanediol the effect of a heavier inoculum was studied. When D-glucose was used as the carbon source, a 5% or 10% inoculum did not show any improvement in the rate or extent of growth (Table 69). No significant difference was observed in diol yields over the range of sugars tested except at a 10% sugar level where more efficient bioconversion took place in the first day of incubation following a heavier inoculum (Table 60 vs 69). The final diol yields, however, were similiar at all inoculum concentrations. Results obtained from cells grown on D-xylose showed the same patterns of response (Table 70). Improvement in cell growth due to heavier inoculum was noted only at 10% xylose levels (Table 70 vs 68). The increase in diol accumulation was demonstrated to be proportional to inoculum size at the initial stages of fermentation. The final diol yields all indicated an inefficient bioconversion process with only about 2% of the sugars utilized for solvent production, even after prolonged incubation (Table 71).

Fed-batch fermentation was attempted as an approach to avoid inhibition due to initial high sugar levels in the medium. Initially, growth and solvent production of the organism proceeded smoothly in media containing 2% glucose (Table 72) or xylose (Table 73), resulting in the accumulation of 0.5% butanediol in 1 day, regardless of the inoculum size. Upon addition of an extra 2% sugar, however, growth and diol production stopped. This lead to a subsequent decrease in the final diol yield

Failure to achieve fed-batch fermentation led us to suspect that either the medium became nutritionally deficient after initial growth and solvent production from the 2% sugar solution, or end-products from such fermentation might have reached inhibitory level and prevented further bioconversion to take place. We therefore grew the organism in 4% sugars with various nutrient supplements. With D-glucose as the carbon source, addition of yeast extract led to an increase in both biomass and butanediol production, resulting in an accumulation of over 1.2% diol (Table 74). An improvement was also obtained when a high ammonium sulfate concentration and trace element level were used, although the increase in diol yield was not as considerable and no increase in biomass could be detected. When urea was added as a supplementary nitrogen source, there was an initial inhibition in both cell growth and diol accumulation. Enhanced diol production subsequently occurred, but the inhibition on biomass yield remained. A similar response to such nutrient supplements was also observed with cultures grown on D-xylose (Table 75).

As these additional nutrients still failed to result in completion of the bioconversion process, we initiated a concurrent program to study inhibition due to end product accumulation during fermentation. Cells were first grown at low sugar concentration in the presence of various end-products in an attempt to acclimatize cells to the inhibitors. A fed-batch system was then used and cell growth and diol production were monitored. When D-glucose was used as the substrate, the presence of ethanol, acetic acid and butanediol all led to a slight inhibition at the initial stage of growth (Table 76). With longer incubation period, however, diol yields all exceeded those obtained from control cultures where no inhibitors were added. Diol production was most enhanced when acetic acid was added, resulting in 3.5 fold increase in values over those obtained in the control. Subsequent addition of yeast extract failed to illicit a further increase in each of these cases.

The same approach was unsuccessful when applied to cells grown on xylose (Table 77). Solvent yields were low in all cultures, including the control containing no inhibitors.

When inhibitors were added to cultures at different times after growth was initiated, the effects were found to be quite different. When D-glucose served as the substrate no apparent inhibition was observed when 0.2% ethanol was added (Table 78). In fact a slight improvement was detected in diol yields, though not in growth (Table 78 vs 76). The addition of acetic acid early in the fermentation process led to inhibition of growth and solvent production (Table 79). The same concentration added later in fermentation subsequently led to enhanced diol accumulation. When butanediol was used, a stimulatory effect was also observed, regardless of the time of addition (Table 80).

When cells were grown on xylose, the effect of end-products was undetermined due to the failure of control cultures to produce solvents when grown under the same fed-batch set up (Table 77). Based on the data obtained, it appeared that ethanol at 0.2% did not result in inhibition (Table 81), acetic acid added early in fermentation led to a decrease in diol production (Table 82), while butanediol addition was actually stimulatory to its own accumulation (Table 83).

Since such effects could be due to the fed-batch system itself, the experiments were repeated with a normal fermentation scheme, using a higher level of end-products. When cells were grown on D-glucose in a medium with various end-products already present, inhibition of growth and diol production was observed (Table 84) particularly when 1.0% acetic acid was present. When ethanol and butanediol were present, cells eventually overcame the inhibition and butanediol production was restored to higher levels than the control level. When D-xylose was used as the carbon source, inhibition of growth occurred with all three end-products, although only acetic acid resulted in lowered diol yields (Table 85).

When the end-products were added to glucose-grown cultures after initiation of growth and fermentation, no inhibition was found upon addition of ethanol (Table 86), acetic acid (Table 87) or butanediol (Table 88), other than an initial retarded diol rate when acetic acid was used. Eventually, cells were able to overcome this inhibitory effect in all cases and increases in butanediol formation resulted, particularly when butanediol was the added end-product.

When the same experiment was carried out with xylose-grown cells, the addition of ethanol (Table 89), acetic acid (Table 90) or butanediol (Table 91) was found to result in enhanced diol yields, reaffirming the fact that the end-products concentration at the levels used (which were already above the levels generally obtained from fermentation) were not inhibitory to growth and solvent production.

The behaviour of cells in the presence of acetic acid appeared erratic, so an attempt was made to test if the inhibitory or stimulatory effects were due to the levels of acetic acid used in the previous experiments. When glucose-grown cells were used, the presence of acetic acid was greatly stimulatory up to a concentration of 0.5% (Table 92) after which inhibition occurred. Acetic acid levels decreased with incubation time, suggesting that it was utilized as well. It is also interesting to note that the final pH of the medium remained around 6.0 when 0.3% or 0.5% acetic acid was added, and an even higher final pH resulted with a 1-2% acetic acid addition, indicating a decrease in acid formation or a rechannelling of acids to ethanol and butanediol. Similar results were obtained with xylose grown cells (Table 93). When neutralized acetic acid was added at a concentration of 0.3-0.5%, diol yields of up to 1.96% and 2.20% could be obtained from a 4.0% glucose or xylose initial concentration, respectively. These values were close to the theoretical yield. Work is now in progress to achieve bioconversion of higher levels of glucose and xylose (up to 20%) either by direct fermentation or by fed-batch systems.

Discussion

Efficient utilization of lignocellulosic wastes requires that most or all of the constituents of biomass substrates be converted to useful products (Bisaria and Ghose, 1981; Flickinger, 1980; Rosenberg, 1980). This is particularly true in the field of energy from renewable resources if the products (liquid fuels) are to be economically competitive with fossil fuels. Research in hemicellulose is therefore crucial in increasing the competitive edge of the bioconversion process. Interest in this area has recently increased considerably, but most work has emphasized only D-xylose utilization (Gong *et al*, 1981; Jeffries, 1981; Schneider *et al*,1981) even though other hexoses and pentoses can account for a significant portion of the total carbohydrate content in hemicellulose (Timell, 1967). To maximize the efficiency of the bioconversion process to result in increased yields, various facultative anaerobes were screened for their abilities to utilize all such sugars for the production of power solvents, *viz*. butanediol, acetone and ethanol. Such a study will then complement our investigation into the pre-treatment of lignocellulosics and bioconversion by obligate anaerobes, thereby providing an overall picture for liquid fuels from hemicellulose.

Aeromonas hydrophilia and *Bacillus macerans* have been reported to carry out butanediol fermentation and acetone-ethanol fermentation, respectively (Rosenberg, 1980). With the five strains of microorganisms tested, significant production of solvents were found only when cells were grown on glucose. In most instances, growth and solvent yields were not detectable in cultures when D-xylose was the sole carbon source. Unless an extensive program of screening, adaptation, acclimatization and/or mutation could be successfully carried out, such organisms did not appear to be ideal candidates for power solvent production from hemicellulose, which contains mostly xylose.

Klebsiella pneumoniae, on the other hand, was shown to be capable of utilizing pentoses (L-arabinose, D-xylose), hexoses (D-galactose, D-glucose and D-mannose) and D-cellobiose, all of which are known to be present in wood hemicellulose (Timell, 1967). Such nutritional versatility suggests that K. pneumoniae could have potential in the utilization of hemicellulose-derived sugars obtained from fractionation of pre-treated wood. The major end-product, 2,3-butanediol, is presently used as an important chemical feedstock for conversion to butadiene, methyl ethyl ketone, etc. As a high grade liquid fuel or fuel additive, it has a heating value of 27 KJ/g, which compares favourably with other liquid fuels (methanol 23 KJ/g, ethanol 29 KJ/g) (Flickinger, 1980). The presence of ethanol as a co-product does not affect the usefulness of butanediol as a fuel, since an equimolar mixture of ethanol and diol can provide a combined heating value of 27.7 KJ/g. Diols are also known to have the additional advantage of being less toxic to microorganism than other solvents, such as n-butanol (Hastings, 1978). This will be significant in future large scale fermentation processes where high yields of butanediol will be encountered.

A detailed study was initiated to optimize butanediol production from K. pneumoniae in order to fully exploit its industrial potential. Although butanediol fermentation had been under intensive research in the

1940's, little recent work has been carried out to optimize the process. Since all 2, 3-butanediol fermentations are sensitive to changes in culture conditions (Long and Patrick, 1963), it is important that a combination of factors be considered simultaneously in defining an optimum condition.

In earlier work dealing with butanediol fermentation, the optimum pH range for the process was suggested to be at 6.0-6.2 (Long and Patrick, 1963; Perlman, 1944). At a more alkaline pH diol yield decreases and diol formation is replaced by organic acid formation. However, if the buffering capacity of the culture media is insufficient, the medium pH rapidly drops to a level where growth and diol accumulation are inhibited (Olson and Johnson, 1948). Therefore, unless automatic or mannal pH titrations are carried out during fermentation, the use of an inert buffer in the media becomes essential. Earlier workers have used excess calcium carbonate as an alternative to pH titration (Long and Patrick, 1963). In our study, however, calcium carbonate at all concentrations tested resulted in a decrease in diol yields. We therefore resorted to MES (pKa 6.1) to provide the necessary buffering capacity. By using a 1.0% MES solution in combination with an initial pH of 6.5, production of 2,3-butanediol was improved when both glucose and xylose were used as substrates. The enhancement in diol yields was probably due to the ability of MES to maintain the culture pH around the optimum for diol production. Higher and lower MES concentration led to a decrease in diol yield, presumably by upsetting the pH balance, resulting in a too alkaline or a too acidic environment for the process. The effectiveness of MES will be tested in the near future in controlled bench-scale fermentors and continuous culture systems as a replacement for pH titration during the entire fermentation.

The optimum temperature range for butanediol fermentation is 30-37°C (Long and Patrick, 1963). This study basically reaffirmed previous findings that fermentation at the higher temperature resulted in increased rate of product formation but with a lower final yield due to a decrease in bioconversion efficiency (McCall and Georgi, 1954).

Production of butanediol is considered to be an anaerobic process (McCall and Georgi, 1954), although earlier workers have noted that aeration or agitation are crucial for maximal diol yield (Adams, 1946; Ledingham and Neish, 1954; Long and Patrick, 1963). Such an effect has been attributed to the removal from the medium of carbon dioxide (McCall and Georgi, 1954), a byproduct in fermentation, which is believed to be detrimental to diol formation (Ledingham and Neish, 1954). In our present study with batch cultures, we have also found the presence of finite air supply in headspace to be essential for optimal production of butanediol. However, by studying the effects of anaerobiosis under various headspace gases, we demonstrated that the presence of 20% CO_2 was beneficial to both growth and diol accumulation, contrary to previous hypothesis (Ledingham and Neish, 1954; McCall and Georgi, 1954). Since growth of _K. pneumoniae_ was favoured in the presence of air, we tested the possibility that decrease in diol yield could be due to a slower growth rate under anaerobic conditions. This possibility was rendered unlikely by our finding that an increase in inoculum size resulted only in an increase in initial fermentation rate but had no effect on final diol yield. Further studies will be required to elucidate the role of limited air supply in butanediol formation.

Under the optimum conditions established so far, complete utilization and bioconversion of a 2% sugar solution of all major monosaccharides present in hemicellulose was achieved with high molar yields of butanediol. However, efficiencies of bioconversion declined sharply with higher sugar concentrations, despite heavier inoculation and fed-batch attempts. We therefore investigated the medium for possible nutrient deficiency (such as an upset of C/N ratio at high sugar level, etc.) and/or buildup of inhibitors. While supplementing the medium with extra nitrogen sources such as yeast extract (also a source of growth factors), urea and ammonium sulfate all tended to improve diol yields, complete utilization of a 4% sugar solution was still not obtained. We then initiated a study of possible end-products which might have accumulated during fermentation to a level inhibitory to butanediol production. Ethanol and butanediol, at concentrations beyond those normally attained during fermentation, were found not to be inhibitory, regardless of the time of introduction to the culture. Acetic acid at high concentration (above 1.0%) was found to inhibit diol accumulation, but at lower levels (0.3-0.5%), acetic acid addition actually resulted in a greatly enhanced production of butanediol. The mechanism of such an activation is at present not well understood, although it could be due to the action of acetic acid on diacetyle (acetoin) reductase, an enzyme which catalyzes the reversible conversion of acetoin to 2, 3-butanediol (Larsen and

Stormer, 1973). An in-depth investigation in this activation mechanism would undoubtedly be crucial to the bioconversion process, especially in view of the fact that acetic acid addition can now result in complete utilization of a concentration of 4% glucose and xylose with a diol yield of 1.96% and 2.20%, respectively. This yield is close to the theoretical yield. We are currently pursuing this exciting aspect by investigating utilization of up to 20% sugars in the presence of acetic acid and/or yeast extract. A successful attempt in this area will definitely bring us one step closer to the industrial application of the process.

To conclude, K. pneumoniae has increasingly been demonstrated as the ideal organism to carry out bioconversion of hemicellulose-derived sugars to butanediol. The present yields and efficiencies surpassed all previous reports (Rosenberg, 1980; Perlman, 1944). Preliminary attempts to utilize wood hydrolyzate have also been successful, reaffirming the great potential the organism possesses. Future research in this area will improve the process even further and significantly affect the economics of producing liquid fuels from lignocellulosics.

REFERENCES

Ackman, R.G. 1972. Porous polymer bead packings and formic acid vapour in the GLC of volatile free fatty acids. J. Chromatogr. Sci. *10*: 560-565

Adams, G.A. 1946. Production and properties of 2,3-butandiol. VII Fermentation of wheat by *Aerobacillus polymyxa* under aerobic and anaerobic conditions. Can. J. Res. *24F*: 1-11

Anderson, R.L. and W.A. Wood 1962. Pathway of L-xylose and L-lysoxe degradation in *Aerobacter aerogenes*. J. Biol. Chem. 237: 296-303.

Bisaria, J.S. and T.K. Ghose 1981. Biodegradation of cellulosic materials, substrates, microorganisms, enzymes and products. Enzyme Microbiol. Technol. *3* 90-104

Clermont, L.P. and N. Schwartz 1952. The chemical composition of Canadian Woods II. Pulp and Paper Mag. Can. *53* 142-143

Compere, A.L. and W.L. Griffiths 1978. Evaluation of substrates for butanol production. IN: Developments in Industrial Microbiology *20* 509-517

Davies, R. and M. Stephenson 1941. Studies on the acetone-butyl alcohol fermentation. Biochem. J. *35*, 1320-1331

Flickinger, M.C. 1980. Current biological research in conversion of cellulosic carbohydrates into liquid fuels: how far have we come? Biotech. Bioeng. *22* Suppl. 1 27-48

Gong, C-S, M.R. Ladisch and G.T. Tsao 1981. Direct fermentation of D-xylose to ethanol by a xylose-fermeting yeast mutant. *Candida* SP XF 217 Biotechnol. Lett. *3* 657-662

Gottschal, J.C. and J.G. Morris 1981. Non-production of acetone and butanol by *Clostridium acetobutylicum* during glucose and ammonium-limitation in continuous culture. Biotechnol. Lett. *3* 525-530

Gottschalk, K.G. and H. Bahl 1981. Feasible improvements of the butanol production by *Clostridium acetobutylicum* in Basic Life Science Vol. 18: Trends in the Biology of Fermentations for Fuels and Chemicals (ed) A. Hollaender. Plenum Press, New York.

Hastings, J.J.H. 1978. Acetone-butyl alcohol fermentation in Economic Microbiology *2* pp. 31-45 ed. A.H. Rose Academic Press, London

Herbert, D., P.J. Phipps and R.E. Strange 1971. Chemical analysis of microbiol cells. pp. 209-344 IN: Methods in Microbiology Vol. 78 J.R. Norris and R.W. Ribbons (eds) Academic Press Inc., New York.

Hungate, R.E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. 14: 1-29

Jeffries, T.W. 1981. Conversion of xylose to ethanol under aerobic conditions by *Candida tropicalis* Biotechnol. Lett. 3 213-218

Larson, S.H. and F.C. Stormer 1973. Diacetyl (acetoin) Reductase from *Aerobacter aerogenes*, kinetic mechanism and regulation by acetate of the reversible reduction of acetoin to 2,3-butanediol. Eur. J. Biochem. 34: 100-106

Ledingham, G.A. and A.C. Neish 1954. Fermentative production of 2,3-butanedio p. 27-93. IN: Industrial Fermentations V. II (ed) L.A. Underkofler and R.J. Kickey. Chemical Publishing Co. Inc., New York

Long, S.K. and R. Patric 1963. The present status of the 2,3-butylene glycol fermentation p. 135-155. In: Advances in Applied Microbiology, Vol. 5 (ed) W.W. Umbriet, Academic Press, New York

Lowenheim, F.A. and M.K. Moran 1975. Butyl alcohol. In: Faith, Keyes and Clark's Industrial Chemicals 4th edition p. 178-185, Wiley-Interscience Toronto

Lowry, O.H., N.R., Rosebrough, A.L. Farr and R.J. Randal 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 93 265-275

Mandels, M., R. Andreotti and C. Roche 1976. Measurement of saccharifying cellulose Biotech. Bioeng. Symp. 6: 21-34

McCall, K.B. and C.E. Georgi 1954. The production of 2,3-butanediol by fermentation of sugar beet molasses. Appl. Microbiol. 2: 355-359

McCutchan, W.N. and R.J. Hickey 1954. The butanol-acetone fermentations In: Industrial Fermentations (eds) L.A. Underkofler and R.J. Hickey. Chemica Publishing Co. New York.

Miller, G.L. 1959. Use of dinitrosalicylic reagent for the determination of reducing sugars. Anal. Chem. 31 426-428

Miller, T.L. and M.J. Wolin 1974. A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. 27: 985-987

Montenecourt, B.S. and D.E. Eveleigh 1977. Semiquantitative plate assay for determination of cellulase production by *Trichoderma viride* Appl. Environ. Microbiol. 33 178-183

Northrop, J.H., L.H. Asha and J.K. Senior 1919. Biochemistry of *Bacillus acetoethylicum* with references to the formation of acetone. J. Biol. Chem. 39 1-21

Ng, T.K., A. Ben-Bassat and J.G. Zeikus 1981. Ethanol production by thermophilic bacteria, fermentation of cellulosic substrates by co-cultures of Clostridium thermocellum and Clostridium thermohydrosulfuricum. Appl. Environ. Microbiol. 41 1337-1343

Olson, B.H. and M.J.J. Johnson 1948. The production of 2,3-butylene glycol by Aerobacter aerogenes 199. J. Bacteriol. 55: 209-222

Perlman, D. 1944. Production of 2,3-butylene glycol from wood hydrolyzates Ind. Agr. Chem. 36: 803-804

Rosenberg, S.L. 1980. Fermentation of pentose sugars to ethanol and other neutral products by microorganisms. Enzyme Microb. Technol. 2: 185-193

Saddler, J.N. 1982. Screening of highly cellulolytic fungi and the action of their cellulase enzyme systems Enzyme and Microbiol. Technology (submitted)

Saddler, J.N., H.H. Brownell, L.P. Clermont and N. Levitin 1982. Enzymatic hydrolysis of cellulose and various pretreated wood fractions. Biotech. Bioeng. 24 (in press)

Schneider, H., P.Y. Wang, Y.K. Chan and R. Maleszka 1981. Conversion of D-xylose into ethanol by the yeast Pachysolen tannophilus Biotechnol. Lett. 3: 89-92

Spivey, J.J. 1978. The acetone/Butanol/ethanol fermentation. Process Biochem. 11: 2-12

Stanier, R.Y. and G.A. Adams 1944. The nature of the Aeromonas fermentation Biochem. J. 38 168-171

Timell, T.E. 1967. Recent progress in the chemistry of wood hemicellulose Wood Sci. Technol. 1 45-70

Van der Lek, J.J. 1930. Onderzoekingen ouer de butylalkoholgisting, Ph.D. thesis, Technische Hoogeschool, Delft

Volesky, B., A. Mulchandani and J. Williams. 1981. Biochemical production of industrial solvents (acetone-butanol-ethanol) from renewable resources. In: Biochemical Engineering II. Annals of the New York Academy of Sciences 369 205-218

Wang, D.I.C., I. Biocie, H-Y. Fang and S-D. Wang. 1979 a Direct microgiological conversion of cellulosic biomass to ethanol pp 61-68 In: 3rd Annual Biomass Energy Systems Conference Proceedings, Colorado

Wang, D.E.C., C.L. Cooney, A.L. Demain, R.F. Gomez and A.J. Sinsky. 1979 b Degradation of cellulosic biomass and its subsequent utilization for the production of chemical feedstocks. U.S. Dept. of Energy Report No. EG775024198 Progress Report from Sept. 1-Nov. 30.

Wang, D.I.C. and H-Y Fang 1980. Ethanol production by a thermophilic and anaerobic bacterium using xylose. In: Proceedings of Am. Chem. Soc. meeting. San Francisco, August

Wang, P.Y., C. Shopsis and H. Schneider 1980. Fermentation of a pentose by yeasts. Biochem. Biophys. Res. Comm. *94* 248-254

Wiegel, J., L.G. Ljungdahl and J.R. Rawson 1979. Isolation from soil and properties of extreme thermophile *Clostridium thermohydrosulfuricum* J. of Bacteriol. *139*: 800-810

Wise, L.E., M. Murphy and A.A. d'Addieco 1946. Chlorite holocellulose, its fractionation and bearing on summatine wood analysis and on studies on the hemicellulose. Paper Trade J. *122* No. 2

Table 1. Lignin, pentosan and hexosan content of various lignocellulosic materials used as substrates[a]

Substrates[b]	Lignin % of total	Pentosans % of total	Hexosans % of total
Xylan (Sigma)	<0.1	79.5	4.1
SEW-WS1 (steam exploded wood water solubles)	11.5	60.4	15.2
SEW-WS2 (steam exploded wood water solubles)	12.2	61.5	16.8
SEW-WS2-0.1%A (Water extract of steam exploded aspen wood soaked in 0.1% H_2SO_4).	17.5	56.3	15.3
SEW-WS2-0.2%A (Water extract of steam exploded aspen wood soaked in 0.2% H_2SO_4).	13.1	51.0	16.1
SEA (Hemicellulose from solvent (alcohol-benzene) extracted aspen)	0.5	85.5	3.8

[a] Values are not additive to 100% because values for ash content, uronic acids, etc were omitted

[b] See materials and methods for full description of substrates

Table 2. Enzyme activities of ethanol precipitated Novo cellobiase

Endoglucanase IU/mg	ß-Glucosidase IU/mg	Filter paper activity IU/mg	Xylanase IU/mg
2.1	10.0	0.2	11.1

Table 3. Sugars released from different pretreated wood substrates after incubation with 1 mg/ml of ethanol precipitated Novo cellobiase[a]

Substrates[b] (20 mg/ml)	Pentosan originally present as % of total	Reducing Sugars (mg/ml)	Monosaccharides (mg/ml)			
			xylose	galatose	glucose	total
Xylan(Sigma)	79.5	11.7	1.5	0.1	0.1	1.5
SEA	85.5	10.8	1.4	0.2	0.1	1.6
SEW-WS2	61.5	9.5	1.2	0.1	0.1	1.2
SEW-WS2-0.1%A	56.3	9.7	1.3	0.1	0.1	1.3
SEW-WS2-0.2%A	51.0	9.9	1.3	0.1	0.1	1.3

[a] Samples were incubated for 3 hr at 50°C and the reaction terminated by placing in a boiling water bath for 10 min.

[b] See materials and methods for full description of substrates

Table 4. Specific cellulase and xylanase activities of culture filtrates from cellulolytic fungi

Enzyme source	Filter paper activity IU/mg	Xylanase activity IU/mg	Original protein concentration in culture supernate (
Trichoderma spp. E58	2.3	132.6	1.6
T. reesei C30	2.6	130.4	2.2
T. viride D39	1.0	59.0	1.0
T. viride D59	2.7	119.1	0.9
Phialophora americana	0.5	11.6	0.5
Scytalidium lignicola	1.1	20.1	0.3
Libertella spp	0.1	2.0	0.1
Myrothecium verrucaria	1.6	50.5	1.5
Novo cellobiase	0.1	5.9	na[a]
Novo celluclast	1.7	8.5	na

[a] not applicable

Table 5. Sugars released from different pretreated wood substrates after incubation with concentrated culture supernates from Trichoderma E58[a]

Substrates[b] (100 mg/ml)	Pentosan originally present as % of total	Reducing Sugars (mg/ml)	Monosaccharides (mg/ml)					
			Xylose	Galactose	Glucose	Mannose	Arabinose	Total
Xylan (sigma)	79.5	58.6	22.8	0.5	4.2	1.6	4.8	34.4
SEA	85.5	56.9	26.3	5.0	<0.1	2.6	0.1	33.9
SEW-WS2	61.5	33.2	23.6	2.7	<0.1	<0.1	<0.1	26.3
SEW-WS2-0.1%A	56.3	34.9	28.5	1.6	<0.1	1.5	<0.1	31.6
SEW-WS2-0.2%A	51.0	42.9	23.2	7.9	5.7	<0.1	<0.1	36.8

[a] 20 ml of each of the substrates at a concentration of 100 mg/ml was incubated for 24 hours at 50°C with 4 ml of Trichoderma E58 at a protein concentration of 8 mg/ml.

[b] See materials & methods for full description of substrates

Table 6: Substrate utilization and ethanol production of *C. thermohydrosulfuricum* on different media

Media	Substrate	Substrate Conc.%	Sugar Used (mg/ml)	Ethanol (mg/ml)	Acetic Acid (mg/ml)
1	Glucose	1	4.6	0.18	0.29
1	Xylose	1	1.5	0.07	0.25
2	Glucose	1	2.7	0.35	0.07
2	Glucose	2	6.0	0.36	0.07
2	Glucose	5	ND	0.33	0.04

1- Media reported by Wiegel J, Ljungdahl L.G. and Rawson J.R. (1979)

2- Modification of media reported by Wang *et al* (1977) media also contained mg/liter in parenthesis $FeCl_3.6H_2O$(6); $MnCl_2.4H_2O$(6); ZnCl(6); $CuSO_4.5H_2O$(6); $CaCl_2$(6)

ND- Not determined

Table 7: Composition of various media used for growth of C. thermosaccharolyticum

Ingredients (g/l)	M1 [a]	M2 [b]	M3 [c]	M4 [d]	M5 [e]	M6 [f]	M7 [g]	M8 [g]	M9 [g]	M10 [g]
KH_2PO_4	1.5	0.75			1.25	1.25	1.5	1.5	1.5	1.5
K_2HPO_4		0.75			0.9					
$Na_2HPO_4.12H_2O$	4.2					0.90	3.0	3.0	3.0	3.0
NH_4Cl	0.5									
NH_4SO_4		2.0	1.0						2.0	2.0
Urea							1.0	1.0		
MgCl	0.18						1.0	1.0		
$MgSO_4$		0.20								
$FeSO_4.7H_2O$							1.25mg	1.25mg	1.25mg	1.25mg
NaCl		1.0								
$CaCO_3$						0.25				
$CaCl_2.2H_2O$	0.05		0.1				0.15	0.15	0.15	0.15
Cysteine hydrochloride		0.5		0.5	0.5	0.5	0.5		0.5	
Sodium thioglycolate								0.5		0.5
Wolfe's mineral elixir	5ml		5ml	5ml						
Vitamin	0.5ml			0.5ml						
Reducing solution	40ml									
Yeast extract (DIFCO)	2.0	5.0	5.0	4.0	1.0	1.0	5.0	5.0	5.0	5.0
Peptone			5.0							
Casein				4.0						
Tryptone					1.0	1.0				

a Wiegel et al, 1979

b Wang et al, 1979b media also contained(mg/L in parenthesis) $FeCl_3.6H_2O(6)$; $MnCl_2.4H_206(6)$; ZnCl(6)g; $CuSO_4.5H_2O(6)$; $CaCl_2.2H_2O(6)$

c Pheil and Ordal, 1967

d Allcock and Woods, 1981

e Lee and Ordal, 1967

f Wilder et al, 1963

g Modifications of media reported by Wang and Fang 1981

Table 8. Test the effect of various media on the glucose utilization and ethanol production of *C*. *thermosaccharolytium*

Media[1]	ATCC Strain	Glucose Used (mg/ml)	Ethanol (mg/ml)	Acetic Acid (mg/ml)	Butyric Acid (mg/ml)
M1	25773	6.3	0.06	0.9	1.18
	7956	7.2	0.23	1.12	1.11
M2	25773	4.9	ND	0.38	1.00
	7956	6.4	ND	0.69	1.01
M3	25773	10.6	0.44	0.71	1.63
	7956	10.5	0.72	0.57	0.57
M4	25773	9.8	0.48	0.55	1.22
	7956	9.7	0.69	0.55	0.63
M5	25773	9.4	0.45	0.31	0.82
	7956	9.7	0.80	0.60	0.71
M6	25773	8.5	ND	0.25	0.81
	7956	10.3	0.91	0.65	1.00
M7	25773	9.6	1.06	1.60	0.98
	7956	9.9	0.79	1.46	1.10
M8	25773	8.9	0.89	1.49	0.97
	7956	9.3	0.86	1.43	1.22
M9	25773	8.4	1.06	1.54	0.99
	7956	8.6	0.83	1.77	1.24
M10	25773	7.4	0.87	1.44	1.04
	7956	8.1	1.02	1.73	1.08

Cultures incubated statically at 60°C for 2 days

ND - not detected

[1] Composition of media described in Table 7

Table 9. Composition of various media used for growth of *C. acetobutylicum*

Ingredients (g/l)	M1 [a]	M2 [b]	M3 [c]	M4 [c]	M5 [c]	M6 [d]	M7 [d]	M8 [e]
KH_2PO_4	1.5	0.75	0.75	0.75	0.75	0.70	0.70	1.0
K_2HPO_4		0.75	0.75	0.75	0.75	0.70	0.70	1.0
$Na_2HPO_4.12H_2O$	4.2							
NH_4Cl	0.5							3.0
NH_4SO_4		2.0	2.0		2.0		0.5	
Asparagine				2.0		0.5		
MgCl	0.18							
$MgSO_4$		0.2	0.2	0.2	0.2	0.10	0.10	0.4
$FeSO_4.7H_2O$			0.01	0.01	0.01	0.015	0.015	
$MnSO_4$			0.01	0.01	0.01	0.10	0.10	
NaCl		1.0	1.0	1.0	1.0	0.01	0.01	
Apteine hydrochloride		0.5	0.5	0.5				
Yeast extract (DIFCO)	2.0	5.0	5.0	5.0	5.0	4.0	4.0	
Biotin								10ug
PABA								10ug
Tryptone						1	1	

a- Wiegel *et al*, 1979; media also contained 5 ml/L Wolfe mineral elixir, 0.5 ml Vitamin mix, 0.05 g $CaCl_2$ and 40 ml/l reducing solution.

b- Media also contained 6 mg/L each of $FeCl_3.6H_2O$, $MnCl_2.4H_2O$, ZnCL; $CuSO_4.5H_2O$, $CaCl_2.2H_2O$

c- Wang *et al*, 1979b

d- Petitdemange *et al*, 1976

e- Gottschal and Morris, 1981

Table 10. Test the effect of media on glucose utilization and solvent production of C. acetobutylicum

Media [a]	Glucose Used (mg/ml)	Solvents (mg/ml)				
		Ethanol	Acetone	Acetic Acid	Butanol	Butyri Aci
M1	7.3	<.05	<.05	0.55	<.05	1.46
M2	17.2	0.82	0.21	0.85	2.22	0.68
M3	19.5	0.43	1.79	0.86	4.61	0.38
M4	19.5	0.44	1.83	0.69	4.60	0.24
M5	19.5	0.36	1.50	1.24	4.22	0.80
M6	19.5	0.33	1.37	1.51	3.67	1.58
M7	19.5	0.38	1.60	1.28	4.41	0.84
M8	5.6	0.13	0.18	0.85	0.81	1.33

Incubated stationary at 37°C for 2 days.

a- Composition of media described in Table 9

Table 11. Test the effect of inoculum age on substrate utilization and solvent production of *C. acetobutylicum*

Inoculum Age (days)	Substrate	Substrate used (mg/ml)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	Glucose	12.0	0.15	0.20	1.55	0.82	1.44
2	Glucose	11.5	0.15	0.26	1.33	0.91	1.70
3	Glucose	12.0	0.14	0.37	1.27	1.19	1.60

Samples were incubated at 37°C with shaking for 2 days.

Table 12. Effect of inoculum size on substrate utilization and solvent production of *C. acetobutylicum*

Inoculum %	O.D. (660 nm)	Glucose used (mg/ml)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Buty Aci
2.5	3.7	19.4	0.42	1.15	1.13	4.61	0.
5.0	4.7	19.4	0.45	1.23	1.25	4.97	0.
10.0	5.0	19.4	0.43	1.12	1.34	4.72	0.
15.0	4.5	19.4	0.40	1.06	1.36	4.54	0.

Incubation at 37°C for 2 days

Table 13: Test the effect of shaking on substrate utilization and solvent production of C. *acetobutylicum*

Inoculum	Shaking[c] (+/-)	Substrate	Substrate used (mg/ml)	Solvents (mg/ml)				
				Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
2-1/2 RCM[a]	+	Glucose	18.8	0.40	1.47	0.95	4.25	0.36
	+	Xylose	6.2	0.21	0.25	1.24	0.36	1.85
	-	Glucose	19.6	0.56	1.21	0.86	4.70	0.34
	-	Xylose	5.8	0.18	0.32	1.14	0.38	1.88
2-1/2 GM[b]	+	Glucose	14.4	0.60	0.19	1.17	0.61	1.75
	+	Xylose	11.7	0.51	0.08	0.91	0.15	1.58
	-	Glucose	15.3	0.68	0.35	0.95	1.65	1.34
	-	Xylose	8.8	0.54	0.06	0.67	0.08	1.28

Incubation at 37°C for 2 days

a- Culture grown on Reinforced Clostridia Media for 2-1/2 days at 37°C

b- Culture grown on the synthetic media of Gottschal and Morris (1981) for 2-1/2 days at 37°C

c- Shaking was done at 175 rpm

Table. 14. Effect of headspace gas on substrate utilization and solvent production of *C. acetobutylicum*

Headspace gas	Substrate Used (mg/ml)	Solvents (mg/ml)				
		Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
N_2	11.2	0.18	0.60	0.99	1.72	1.35
N_2:CO_2 80:20	8.9	0.14	0.54	1.04	1.41	1.65
H_2	17.2	0.26	0.91	0.77	2.80	1.00
H_2:CO_2 80:20	15.0	0.22	0.82	0.82	2.29	1.07

Incubation at 37°C for 2 days under stationary conditions

Table 15: Effect of yeast extract on substrate utilization and solvent production of *C. acetobutylicum*

Yeast Extract (g/l)	Substrate 2%	O.D (660 nm)	Substrate used (mg/ml)	Solvents (mg/ml)				
				Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
0	Glucose	0.55	<0.5	<.05	0.21	0.51	0.34	0.52
1.25	Glucose	1.20	3.5	0.07	0.23	0.83	0.62	1.01
2.50	Glucose	2.64	9.6	0.15	0.64	0.91	1.99	0.67
3.75	Glucose	4.36	19.0	0.38	1.19	0.72	3.62	0.32
5.00	Glucose	5.58	19.4	0.42	1.22	1.02	3.92	0.41
0	Xylose	0.60	<0.5	<.05	<.05	0.58	0.12	0.84
1.25	Xylose	0.90	0.6	0.06	0.11	0.79	0.45	1.39
2.50	Xylose	1.20	1.4	0.08	0.13	1.13	0.57	2.04
3.75	Xylose	1.90	5.7	0.14	0.08	1.33	0.48	2.90
5.00	Xylose	1.80	7.7	0.13	0.08	1.30	0.46	2.92

Incubation at 37°C for 2 days under stationary conditions

Table 16. Utilization of substrate and production of solvents by *C. acetobutylicum* with time

Incubation Time (hours)	Substrate (2%)	Substrate used (mg/ml)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
0	Glucose	0.0	<.05	<.05	<.05	<.05	0.23
4	Glucose	0.2	0.39	0.08	0.45	<.05	0.20
24	Glucose	4.1	0.19	0.10	0.81	0.60	1.78
48	Glucose	10.1	0.27	0.37	1.13	2.73	1.14
72	Glucose	10.1	0.49	0.85	1.22	2.83	1.15
144	Glucose	10.1	0.29	0.46	1.21	2.92	1.45
0	Xylose	0.0	0.11	<.05	<.05	<.05	0.26
4	Xylose	0.4	0.36	0.07	0.23	0.10	0.41
24	Xylose	3.0	0.16	0.06	0.61	0.12	1.35
48	Xylose	10.9	0.24	0.13	1.24	0.85	2.31
72	Xylose	10.9	0.41	0.43	1.28	0.87	2.21
144	Xylose	10.9	0.25	0.12	1.37	0.91	2.72

Incubation at 37°C under stationary conditions

Table 17a. Effect of surface/volume ratio on glucose utilization and solvent production of *C. acetobutylicum*

Vial Size (mls)	Media mls	s/v^a	Glucose used (mg/ml)	Solvents (mg/ml)				
				Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
10	2	1.63	19.4	0.53	0.84	1.32	3.03	1.14
	4	0.81	19.2	0.31	0.89	1.28	3.06	1.07
	5	0.65	16.5	0.28	0.80	1.08	2.68	0.94
	6	0.54	17.0	0.56	0.77	1.14	2.67	1.02
	8	0.41	14.2	0.24	0.56	1.09	2.21	1.06
60	5	2.13	19.3	0.39	0.99	1.28	2.83	1.01
	10	1.07	18.6	0.26	0.96	1.28	2.77	1.17
	20	0.53	14.9	0.22	0.59	1.17	2.14	1.23
	25	0.43	12.7	0.18	0.45	1.09	1.35	1.31
	30	0.36	11.2	0.17	0.33	1.04	1.51	1.37
	40	0.27	11.2	0.15	0.26	0.96	1.25	1.38
	50	0.22	14.2	0.20	0.43	1.10	1.79	1.22
160	25	0.69	19.3	0.29	0.83	1.15	2.76	1.03
	50	0.34	15.1	0.28	0.72	0.97	2.52	0.94
	75	0.23	9.8	0.23	0.54	0.94	2.09	0.97
	100	0.17	8.3	0.19	0.37	0.88	1.65	1.06
	125	0.14	8.1	0.18	0.33	0.84	1.49	1.08
	150	0.11	7.5	0.17	0.28	0.78	1.28	1.04

Incubation at 37°C for 2 days

a- Surface of each vial was calculated using inside diameter; the surface values for each vial were: 3.25 mm^2 (10 ml), 10.67 mm^2 (60 ml), 17.15 mm^2 (160 ml)

Table 17b. Effect of surface/volume ratio on xylose utilization and solvent production of *C. acetobutylicum*

Vial Size (mls)	Media mls	s/v[a]	Xylose used (mg/ml)	Solvents (mg/ml)				
				Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
10	2	1.63	7.5	0.13	0.09	1.33	0.28	2.50
	4	0.81	4.8	0.14	0.07	1.19	0.51	2.38
	6	0.54	4.6	0.14	0.05	1.04	0.47	2.18
	8	0.41	5.0	0.13	0.05	1.01	0.48	2.12
60	5	2.13	7.0	0.12	0.13	1.25	0.56	2.61
	10	1.07	6.4	0.13	0.08	1.14	0.51	2.42
	25	0.53	4.8	0.13	0.05	1.03	0.44	2.20
	30	0.36	4.2	0.14	0.04	0.97	0.41	2.05
	40	0.27	3.2	0.18	0.06	1.05	0.61	2.00
	50	0.22	3.4	0.17	0.05	0.98	0.59	1.87
160	25	0.69	8.2	0.20	0.10	1.27	0.81	2.43
	50	0.34	5.6	0.19	0.07	1.18	0.76	2.20
	75	0.23	2.6	0.12	0.03	0.85	0.30	1.81
	100	0.17	5.4	0.18	0.04	0.99	0.55	1.93
	125	0.14	4.2	0.16	0.04	0.93	0.51	1.80
	150	0.11	3.6	0.16	0.05	0.84	0.51	1.59

Incubation at 37°C for 2 days

a- Surface of each vial was calculated using inside diameter; the surface values for each vial were: 3.25 mm^2 (10 ml), 10.67 mm^2 (60 ml), 17.15 mm^2 (160 ml)

Table 18. Substrate utilization and solvent production of *C. acetobutylicum* grown on enzymatically hydrolyzed wood hemicellulose fractions[a]

Substrates (10 g/l)	Reducing Sugars Detected after Enzyme Hydrolysis (g/l)	%of Carbon Detected as Reducing Sugars Utilized	Solvent (g/l)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
Xylan (Sigma)	5.99	87	<.05	0.08	0.33	0.21	0.41
Steam-exploded wood; water solubles	3.67	91	<.05	0.04	0.95	0.45	0.52
Solvent extracted aspen	5.64	73	<.05	0.05	1.84	0.18	0.23

Incubation at 37°C for 2 days

a- These fractions were hydrolyzed by *Trichoderma* E58 cellulase

Table 19: Substrate utilization and solvent production of *C. acetobutylicum* using sugars normally found in hemicellulose hydrolysates as substrates

Substrate	Substrate used (mg/ml)	O.D. (660 nm)	Solvents (mg/ml)					Butanol Yield (g butanol/ g substrate)
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid	
Glucose	19.5	5.12	0.32	1.20	0.85	4.08	0.49	0.21
Cellobiose	18.7	3.63	0.40	1.13	0.71	4.21	0.41	0.23
Mannose	16.9	3.27	0.29	0.89	1.30	3.10	1.08	0.18
Arabinose	10.8	2.79	0.13	0.53	1.81	0.98	1.43	0.09
Xylose	12.2	2.56	0.16	0.28	1.22	0.87	2.15	0.07
Galactose	11.6	2.66	0.16	0.15	1.08	0.25	2.28	0.02

Incubation at 37°C for 2 days

Table 20. The effect of various concentrations of glucose on growth and solvent production of *C. acetobutylicum*

Glucose Conc.(%)	Incubation Time (days)	O.D. (660 nm)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	2	3.02	0.31	0.24	1.32	1.19	1.64
1	4	2.64	0.18	0.32	1.47	1.30	1.36
2	2	5.12	0.52	1.53	1.00	4.65	0.52
2	4	4.05	0.43	1.36	1.06	4.17	0.53
3	2	5.36	0.57	1.82	0.68	5.67	0.49
3	4	4.58	0.50	1.70	0.77	5.01	0.52
5	2	4.98	0.42	1.44	0.94	4.22	0.68
5	4	4.13	0.47	1.47	0.89	4.34	0.56
10	2	4.01	0.28	1.05	0.99	3.22	0.83
10	4	3.47	0.30	1.33	1.04	3.32	0.86

Incubation at 37°C

Table 21. The effect of various concentrations of xylose on growth and solvent production of *C. acetobutylicum*

Xylose Conc. (%)	Incubation Time (days)	O.D. (660 nm)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	2	2.10	0.22	0.07	0.91	0.26	1.75
1	4	1.85	0.25	0.19	1.18	0.32	1.89
2	2	2.56	0.10	0.10	1.04	0.44	2.19
2	4	2.09	0.27	0.20	1.15	0.42	2.16
3	2	2.53	0.11	0.13	1.08	0.57	2.19
3	4	2.21	0.24	0.21	1.10	0.64	2.20
5	2	2.49	0.24	0.24	1.06	0.71	2.18
5	4	2.07	0.11	0.30	1.14	0.89	2.14
10	2	1.28	0.21	0.10	0.67	0.24	1.14
10	4	1.59	0.19	0.41	1.16	1.43	1.51

Incubation at 37°C

Table 22. The effect of various concentrations of mannose on growth and solvent production of *C. acetobutylicum*

Mannose Conc. (%)	Incubation Time (days)	O.D. (660 nm)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	2	3.03	0.10	0.14	1.28	0.59	2.36
1	4	2.50	0.13	0.20	1.14	0.66	2.24
2	2	3.27	0.37	0.28	1.33	1.16	2.28
2	4	2.62	0.30	0.26	1.21	0.89	2.19
3	2	3.06	0.14	0.20	1.27	0.93	1.88
3	4	2.59	0.23	0.29	1.30	0.94	2.08
5	2	3.08	0.23	0.32	1.42	1.46	1.86
5	4	2.68	0.38	0.37	1.64	1.36	1.92
10	2	2.73	0.31	0.26	1.36	1.14	1.97
10	4	1.43	0.18	0.30	1.11	1.18	1.99

Incubation at 37°C

Table 23. The effect of various concentrations of arabinose on growth and solvent production of *C. acetobutylicum*

Arabinose Conc. (%)	Incubation Time (days)	O.D. (660 nm)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	2	2.80	0.50	0.31	2.18	0.58	1.67
1	4	2.43	0.18	0.32	2.17	0.66	1.64
2	2	2.79	0.40	0.38	1.91	0.79	1.56
2	4	2.46	0.34	0.44	2.15	0.87	1.61
3	2	2.80	0.45	0.49	1.82	0.92	1.45
3	4	2.60	ND[a]	ND[a]	ND[a]	ND[a]	ND[a]
5	2	3.09	0.21	0.91	1.57	1.77	0.94
5	4	3.34	0.33	1.42	1.64	3.02	0.86
10	2	2.79	0.34	1.41	1.13	2.57	0.43
10	4	1.43	0.44	1.78	1.11	3.27	0.40

Incubation at 37°C

a- not determined

Table 24. The effect of various concentrations of galactose on growth and solvent production of *C. acetobutylicum*

Galactose Conc. (%)	Incubation Time (days)	O.D. (660 nm)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	2	2.33	0.51	0.28	0.85	0.32	1.64
1	4	2.10	0.14	0.16	1.31	0.31	2.13
2	2	2.67	0.41	0.13	1.03	0.46	2.20
2	4	2.21	0.17	0.11	1.29	0.31	2.38
3	2	2.51	0.46	0.12	0.94	0.61	1.97
3	4	2.20	1.11	0.17	1.07	0.28	2.34
5	2	2.26	0.82	0.12	0.87	0.71	1.88
5	4	2.24	0.13	0.15	1.29	0.31	2.39
10	2	1.96	0.48	0.11	0.86	0.75	2.08
10	4	2.00	0.16	0.15	1.03	0.38	2.41

Incubation at 37°C

Table 25. The effect of various concentrations of cellobiose on growth and solvent production of *C. acetobutylicum*

Cellobiose Conc. (%)	Substrate Used (mg/ml)	O.D. (660 nm)	Solvents (mg/ml)				
			Ethanol	Acetone	Acetic Acid	Butanol	Butyric Acid
1	9.6	3.09	0.15	0.41	1.13	1.39	1.49
2	19.5	3.63	0.43	1.34	0.74	3.84	0.46
3	28.6	5.20	0.81	1.99	0.43	5.68	0.32
5	30.0	5.07	0.82	2.12	0.42	6.00	0.28
10	41.5	4.14	0.86	2.14	0.42	5.76	0.13

Incubation for 2 days at 37°C

Table 26. Growth and solvent production of *A. hydrophilia* under anaerobic conditions.[a]

Strain	Medium	C source	% C utilized	O.D. 540 (nm)	Final pH	Solvents (mg/L)			
						Ethanol	Acetic Acid	Butanediol	Total
NRCC 1001	NB[b]	n/a[c]	n/a	0.41	7.21	ND[d]	ND	ND	ND
	NB	1% glucose	68.6	1.01	5.31	946.4	124.3	1383.8	2472.5
	Ah[e]	1% xylose	0.0	0.02	7.44	ND	ND	ND	ND
	Ah	1% glucose	56.6	0.58	5.50	294.6	71.5	310.7	676.8
NRCC 1002	NB	n/a	n/a	0.03	7.48	ND	ND	ND	ND
	NB	1% glucose	65.5	0.90	5.36	432.2	344.8	371.4	1184.4
	Ah	1% xylose	0.0	0.02	7.40	ND	ND	ND	ND
	Ah	1% glucose	49.0	0.27	6.58	ND	ND	ND	ND

a- Culture grown anaerobically (N_2 atmosphere) at 30°C (150 rpm shaking) for 4-1/2 days.
b- Nutrient broth (Difco)
c- Not applicable
d- Not detected
e- Minimal medium for *A. hydrophilia*

Table 27. Growth and solvent production of *B. macerans* under anaerobic conditions.[a]

Strain	Medium	C source	% C utilized	O.D. 540 (nm)	Final pH	Solvents (mg/L)			
						Ethanol	Acetic Acid	Butanediol	Total
NRCC 9073	NB[b]	n/a[c]	n/a	0.03	7.40	ND[d]	ND	ND	ND
	NB	1% glucose	0.0	0.02	7.42	ND	ND	ND	ND
	BM[e]	1% xylose	0.0	0.02	7.36	ND	ND	ND	ND
	BM	1% glucose	53.0	0.47	6.50	ND	ND	ND	ND
NRCC 9074	NB	n/a	n/a	0.03	7.40	ND	ND	ND	ND
	NB	1% glucose	40.7	0.39	5.45	186.1	ND	314.3	500.4
	BM	1% xylose	0.0	0.02	7.31	ND	ND	ND	ND
	BM	1% glucose	84.0	0.98	5.73	1050.3	330.4	1733.2	3113.9
NRCC 9075	NB	n/a	n/a	0.04	7.42	ND	ND	ND	ND
	NB	1% glucose	0.0	0.02	7.40	ND	ND	ND	ND
	BM	1% xylose	0.0	0.02	7.30	ND	ND	ND	ND
	BM	1% glucose	46.5	0.19	6.85	ND	ND	ND	ND

a- Cultures were grown under N_2 at 30°C with shaking (150 rpm) for 4-1/2 days
b- Nutrient broth
c- Not applicable
d- Not detected
e- Minimal medium for *B. macerans*

Table 28. Solvent production by A. hydrophilia and B. macerans grown anaerobically[a]

Organism	Strain	Medium	C source	Solvents (mg/L)					
				Ethanol	Acetone	Acetic Acid	Butanol	Butanediol	Total
A. hydrophilia	NRCC 1001	AL[b]	1% xylose	ND[c]	12.3	151.2	4.6	ND	168.1
	NRCC 1002	AL	1% xylose	ND	7.7	54.6	14.6	ND	76.9
B. macerans	NRCC 9073	BM[d]	2% xylose	ND	11.0	70.1	9.9	ND	91.0
	NRCC 9074	BM	2% xylose	847.4	291.5	970.5	10.8	260.4	2380.6
	NRCC 9075	BM	2% xylose	ND	ND	67.7	4.7	ND	72.4

a- Cultures were grown under N_2 at 30°C with shaking (150 rpm) for 30 days
b- Minimal medium for A. hydrophilia
c- Not detected
d- Minimal medium for B. macerans

Table 29. Solvent production of *A. hydrophilia* under aerobic conditions.[a]

Strain	Medium	C source	% C utilized	Solvents (mg/L)		
				Acetic Acid	Butanediol	Total
NRCC 1001	NB[b]	1% glucose	83.5	ND[c]	146.2	146.2
	NB	1% xylose	5.6	ND	ND	ND
	AH[d]	1% glucose	87.5	27.7	520.2	547.9
	AH	1% xylose	0.0	20.4	ND	20.4
NRCC 1002	NB	1% glucose	88.2	254.8	925.7	1142.2
	NB	1% xylose	0.0	ND	ND	ND
	AH	1% glucose	81.3	ND	ND	ND
	AH	1% xylose	7.6	ND	ND	ND

a- Cultures were grown aerobically at 30°C with shaking (150 rpm) for 5 days
b- Nutrient broth
c- Not detected
d- Minimal medium for *A. hydrophilia*

Table 30. Solvent production of B. macerans grown aerobically.[a]

Strain	Medium	C source	% C utilized	Solvents (mg/L)		
				Acetic Acid	Butanediol	Total
NRCC 9073	NB[b]	1% glucose	6.6	335.6	ND[c]	335.6
	NB	1% xylose	21.0	ND	ND	ND
	BM[d]	1% glucose	29.6	312.8	ND	312.8
	BM	1% xylose	10.0	ND	ND	ND
NRCC 9074	NB	1% glucose	17.0	209.5	ND	209.5
	NB	1% xylose	15.6	75.8	ND	75.8
	BM	1% glucose	11.0	155.3	ND	155.3
	BM	1% xylose	1.0	ND	ND	ND
NRCC 9075	NB	1% glucose	6.6	ND	ND	ND
	NB	1% xylose	10.0	ND	ND	ND
	BM	1% glucose	11.6	532.5	ND	532.5
	BM	1% xylose	0.0	ND	ND	ND

a- Cultures were grown aerobically at 30°C with shaking (150 rpm) for 5 days
b- Nutrient broth
c- Not detected
d- Minimal medium for B. macerans

Table 31. Growth and solvent production of *Klebsiella pneumoniae* in various media.[a]

Medium	C source	% C utilized	O.D. (540 nm)	Solvents (mg/L)			
				Ethanol	Acetic Acid	Butanediol	Total
NB[b]	n/a[c]	n/a	0.27	0.06	0.08	ND[d]	0.14
	glucose	72.5	0.64	1.39	0.64	1.34	3.37
	xylose	50.8	0.47	1.08	0.92	0.36	2.36
Kp[e]	glucose	100.0	1.81	1.50	0.09	3.32	4.91
	xylose	50.5	1.39	0.78	0.59	0.56	1.93

a- Cultures were incubated anaerobically (N_2) at 37°C without shaking for 4-1/2 days
b- Nutrient broth
c- Not applicable
d- Not detected
e- Minimal medium for *K. pneumoniae*

Table 32. Solvent production by *K. pneumoniae* grown aerobically and anaerobically under shake-flask conditions.[a]

C Source[b]	Growth Condition	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
L-arabinose	O_2	4.67	6.26	100.0	0.18	0.92	ND[c]	1.10
	N_2	1.09	5.13	100.0	1.34	0.30	2.50	4.14
D-xylose	O_2	4.12	6.06	100.0	0.11	0.78	ND	0.89
	N_2	0.84	5.17	57.5	0.96	0.76	0.46	2.18
D-glucose	O_2	4.30	5.90	100.0	0.30	0.45	0.23	0.98
	N_2	1.41	5.31	100.0	1.77	0.14	3.43	5.34
D-galactose	O_2	6.04	6.60	100.0	0.07	0.20	ND	0.27
	N_2	1.15	5.43	100.0	1.36	0.26	2.26	3.88
D-mannose	O_2	5.22	6.67	100.0	0.02	ND	ND	0.02
	N_2	1.27	5.28	100.0	1.49	0.28	2.63	4.40
D-cellobiose	O_2	5.17	6.62	100.0	0.09	ND	ND	0.09
	N_2	1.11	5.26	72.2	1.19	0.54	0.95	2.68

a- Cultures were grown in Kp medium and incubated at 37°C with shaking (150 rpm) for 24 hours

b- 1% solution (final concentration)

c- Not detected

Table 33. Solvent production by *K. pneumoniae* grown aerobically and anaerobically under non-shaking conditions.[a]

C Source[b]	Growth Condition	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
L-arabinose	O_2	5.48	6.60	100.0	0.12	0.22	0.09	0.43
	N_2	0.91	5.10	78.5	1.15	0.25	1.35	2.75
D-xylose	O_2	4.12	6.45	100.0	0.48	0.53	1.10	2.11
	N_2	0.84	5.00	46.0	0.84	0.62	0.27	1.73
D-glucose	O_2	5.96	6.50	100.0	0.45	0.22	0.27	0.94
	N_2	0.95	4.90	85.5	1.00	0.18	1.20	2.38
D-galactose	O_2	4.12	6.00	100.0	0.11	0.18	0.07	0.36
	N_2	0.91	5.15	58.3	1.19	0.10	1.91	3.20
D-mannose	O_2	5.58	6.60	100.0	0.10	0.24	0.27	0.61
	N_2	0.98	4.85	77.0	0.99	0.26	1.36	2.61
D-cellobiose	O_2	5.15	6.60	100.0	0.15	0.21	0.08	0.44
	N_2	0.81	5.10	48.5	0.76	0.40	0.46	1.62

a- Cultures were grown in Kp medium and incubated at 37°C without shaking for 3 days

b- 1% solution (final concentration)

Table 34. Solvent production by *K. pneumoniae* grown anaerobically on various glucose concentrations under shaking conditions.[a]

Glucose Conc. %	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L) Ethanol	Solvents (g/L) Acetic Acid	Solvents (g/L) Butanediol	Total
1	0.96	5.31	81.8	1.42	ND[b]	3.29	4.71
2	1.05	5.28	36.0	1.42	ND	3.57	4.99
3	1.14	5.13	30.0	1.46	ND	3.57	5.03
4	1.06	5.12	21.5	1.47	ND	3.72	5.19
6	1.07	5.07	24.7	1.38	ND	3.92	5.30
8	1.03	5.10	28.8	1.45	0.05	3.98	5.48
10	0.84	4.99	23.9	1.17	ND	3.73	4.90

a- Cultures were incubated in Kp medium at 37°C with shaking (150 rpm) for 3 days
b- Not detected

Table 35. Solvent production by *K. pneumoniae* grown anaerobically on various xylose concentrations under shaking conditions.[a]

Xylose Conc. %	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L) Ethanol	Solvents (g/L) Acetic Acid	Solvents (g/L) Butanediol	Solvents (g/L) Total
1	0.67	5.17	45.0	1.09	0.51	0.42	2.02
2	0.76	5.03	39.0	0.65	0.35	1.55	2.55
3	0.78	4.92	28.3	0.91	0.23	1.65	2.79
4	0.86	4.87	32.0	0.90	0.16	1.82	2.88
6	0.86	5.00	25.0	1.02	0.18	2.21	3.41
8	0.84	4.86	21.3	0.90	0.19	2.06	3.15
10	0.71	4.81	10.4	0.76	0.16	1.89	2.81

a- Cultures were incubated in Kp medium at 37°C with shaking (150 rpm) for 3 days

Table 36. Solvent production by *K. pneumoniae* grown anaerobically on various glucose concentrations under non-shaking conditions.[a]

Glucose Conc. %	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
1	0.95	4.9	60.0	1.00	0.18	1.20	2.38
2	1.06	4.9	40.5	1.24	0.10	1.85	3.19
3	1.04	4.9	25.8	1.29	0.06	2.18	3.53
4	1.03	4.7	17.9	1.00	0.08	1.58	2.66
6	1.03	4.7	19.7	0.87	0.26	1.30	2.43
8	0.94	4.6	20.9	0.74	0.24	0.73	1.71
10	0.92	4.5	18.0	0.59	0.26	0.43	1.28

a- Cultures were incubated in Kp medium at 37°C without shaking for 7 days

Table 37. Solvent production by *K. pneumoniae* grown anaerobically on various xylose concentrations under non-shaking conditions.[a]

Xylose Conc. %	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
1	0.84	5.0	46.0	0.84	0.62	0.27	1.73
2	0.95	5.0	46.0	1.00	0.23	1.26	2.49
3	0.93	5.4	38.0	1.26	0.04	2.19	3.49
4	0.93	5.0	25.0	1.01	0.04	1.67	2.72
6	0.93	4.9	29.6	1.01	0.10	1.61	2.72
8	0.98	4.8	21.9	0.79	0.26	0.96	2.01
10	0.96	4.7	20.5	0.67	0.29	0.61	1.57

a- Cultures were incubated in Kp medium at 37°C without shaking (150 rpm) for 7 days

Table 38. Solvent production by *K. pneumoniae* grown anaerobically on various glucose concentrations.[a]

Glucose Conc. %	Inoculation Time (days)	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L) Ethanol	Acetic Acid	Butanediol	Total
0.1	1	0.38	6.81	100.0	0.27	0.26	ND[b]	0.53
	2	0.35	6.89	100.0	0.31	0.25	ND	0.56
0.2	1	0.66	6.57	100.0	0.53	0.56	ND	1.09
	2	0.58	6.62	100.0	0.54	0.44	ND	0.98
0.3	1	0.80	6.14	100.0	0.44	0.90	ND	1.34
	2	0.73	6.18	100.0	0.68	0.61	ND	1.29
0.5	1	0.91	5.55	100.0	0.56	0.95	0.45	1.96
	2	0.94	5.54	100.0	0.93	0.66	0.55	2.14
1.0	1	1.10	5.32	91.6	0.90	0.65	3.29	4.84
	2	1.21	5.32	100.0	1.75	0.32	3.13	5.20
2.0	1	1.12	5.28	47.0	1.02	0.65	3.76	5.43
	2	1.21	5.36	84.0	1.88	0.02	6.70	8.60
3.0	1	1.04	5.23	27.5	1.01	0.67	2.15	3.83
	2	1.16	5.31	43.3	2.13	0.02	6.73	8.88
4.0	1	0.89	5.14	19.1	1.06	0.75	1.19	3.00
	2	0.99	4.98	26.3	1.58	0.25	3.43	5.26
6.0	1	0.86	5.10	10.6	1.05	0.79	1.18	3.02
	2	0.99	4.86	26.2	1.29	0.43	2.35	4.07
8.0	1	0.73	5.15	17.9	1.04	0.68	0.78	2.50
	2	0.87	4.77	23.4	1.05	0.61	1.47	3.13
10.0	1	0.79	4.97	18.0	1.15	0.63	1.51	3.29
	2	0.93	4.72	22.2	1.15	0.52	2.12	3.79

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm) under N_2

b- Not detected

Table 39. Solvent production by *K. pneumoniae* grown anaerobically on various concentrations of xylose.[a]

Xylose Conc. %	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
0.1	0.28	6.96	100.0	0.36	0.12	ND[b]	0.48
0.2	0.41	6.60	63.5	0.53	0.43	ND	0.96
0.3	0.50	6.26	67.3	0.63	0.80	ND	1.43
0.5	0.59	5.70	80.2	0.80	0.93	0.25	1.98
1.0	0.56	5.58	51.8	0.88	0.90	0.46	2.24
2.0	0.67	5.42	38.0	0.98	0.84	0.43	2.25
3.0	0.76	5.21	30.0	0.84	0.91	0.69	2.44
4.0	0.80	5.08	26.0	1.04	0.72	0.66	2.42
6.0	0.83	4.87	28.6	0.79	0.72	0.74	2.25
8.0	0.84	4.89	43.5	0.71	0.85	0.66	2.22
10.0	0.76	5.00	22.2	0.54	0.68	0.70	1.92

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm) under N_2 for 2 days

b- Not detected

Table 40. Solvent production by *K. pneumoniae* grown anaerobically on glucose for extended periods.[a]

Glucose Conc. %	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
0.5	14	0.82	5.56	0.98	0.67	0.60	2.25
	50	0.91	5.57	0.37	0.79	0.42	1.68
1.0	14	1.18	5.35	1.74	0.37	2.80	4.91
	50	1.32	5.32	1.57	0.64	2.17	4.38
2.0	14	1.19	5.36	2.17	0.02	5.43	7.62
	50	1.26	5.34	2.05	0.41	3.79	6.25
3.0	14	1.14	5.30	1.75	0.02	5.10	6.87
	50	1.21	5.30	1.91	0.62	3.56	6.09
4.0	14	1.04	4.99	1.74	0.29	2.87	4.90
	50	1.20	4.96	1.55	0.81	2.24	4.60
6.0	14	1.04	4.88	1.52	0.49	2.04	4.05
	50	1.21	4.88	1.40	0.88	1.50	3.78
8.0	14	0.91	4.80	1.34	0.64	0.86	2.84
	50	0.92	4.74	1.19	0.90	0.90	2.99
10.0	14	0.98	4.75	1.43	0.57	1.40	3.40
	50	1.05	4.70	1.10	0.81	1.64	3.55

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm) under N_2

Table 41. Solvent production by *K. pneumoniae* grown anaerobically on D-xylose for extended periods.[a]

Xylose Conc. %	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
0.5	14	0.55	5.57	1.00	0.97	ND[b]	1.97
	50	0.51	5.60	0.82	0.93	0.17	1.92
1.0	14	0.62	5.31	1.02	0.88	ND	1.90
	50	0.60	5.30	0.95	0.89	0.07	1.91
2.0	14	0.74	5.12	1.15	0.82	0.51	2.48
	50	0.77	5.16	1.08	0.88	0.35	2.31
3.0	14	0.83	5.07	1.21	0.77	0.54	2.52
	50	0.92	5.09	1.14	0.86	0.44	2.44
4.0	14	0.85	4.97	1.05	0.77	0.70	2.52
	50	0.99	4.99	1.00	0.98	0.41	2.39
6.0	14	0.88	4.89	0.73	0.85	0.47	2.05
	50	0.91	4.90	0.92	0.94	0.25	2.11
8.0	14	0.87	4.83	0.96	0.70	0.66	2.35
	50	0.93	4.87	0.82	0.79	0.34	1.95
10.0	14	0.84	4.86	0.88	0.57	0.62	2.07
	50	0.91	4.89	0.70	0.71	0.33	1.74

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm) under N_2

b- Not detected

Table 42. Solvent production by _K_. _pneumoniae_ grown anaerobically under N_2:CO_2 (80:20).[a]

Substrates[b]	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
L-arabinose	1	0.86	5.41	1.34	0.64	1.52	3.50
	7	1.01	5.29	1.72	0.54	1.77	4.03
D-xylose	1	0.99	5.23	1.14	0.82	0.78	2.74
	7	1.19	5.18	1.28	0.86	0.47	2.61
D-glucose	1	0.76	5.37	1.08	0.65	1.47	3.20
	7	1.08	5.45	1.93	0.01	3.30	5.24
D-galactose	1	0.90	5.37	1.05	0.72	1.40	3.17
	7	1.13	5.28	1.59	0.59	1.80	3.98
D-mannose	1	0.79	5.20	0.98	0.86	0.39	2.23
	7	1.13	5.06	1.29	0.48	0.55	2.32
D-cellobiose	1	1.11	5.27	0.89	1.01	0.39	2.29
	7	1.19	5.24	1.24	0.81	0.28	2.34

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm)

b- 1% sugar (final concentration)

Table 43. Solvent production by K. pneumoniae grown anerobically under H_2:CO_2 (80:20).[a]

Substrates[b]	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
L-arabinose	1	0.85	5.46	1.14	0.66	1.46	3.26
	7	0.98	5.28	1.76	0.19	2.69	4.64
D-xylose	1	0.98	5.26	1.07	0.92	0.62	2.61
	7	1.20	5.19	1.25	0.85	0.40	2.50
D-glucose	1	0.86	5.34	1.26	0.71	1.41	3.38
	7	1.00	5.50	1.92	0.01	3.33	5.26
D-galactose	1	0.82	5.40	1.20	0.65	1.60	3.45
	7	1.02	5.27	1.68	0.24	2.24	4.16
D-mannose	1	0.96	5.20	1.08	0.94	0.51	2.53
	7	1.05	5.08	1.23	0.44	0.50	2.17
D-cellobiose	1	1.09	5.27	1.14	0.89	0.51	2.54
	7	1.18	5.21	1.29	0.91	0.28	2.48

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm)

b- 1% sugar (final concentration)

Table 44. Solvent production by *K. pneumoniae* grown aerobically.[a]

Substrates[b]	Shaking	Incubation Time	O.D. (540 nm)	Final pH	%C utilized	Solvents (g/L)			
						Ethanol	Acetic Acid	Butanediol	Total
D-glucose	0	18 h	1.99	5.93	95.9	0.76	0.06	2.42	3.24
		4-1/2 d	6.19	6.73	100.0	ND[c]	ND	ND	ND
	150 rpm	18 h	5.70	6.30	94.9	0.18	0.54	0.48	1.20
		4-1/2 d	5.42	6.68	100.0	ND	ND	ND	ND
D-Xylose	0	18 h	1.71	5.68	68.5	0.84	0.58	1.27	2.69
		4-1/2 d	5.32	6.68	100.0	ND	ND	ND	ND
	150 rpm	18 h	5.00	6.35	100.0	0.52	0.24	ND	0.76
		4-1/2 d	5.28	6.63	100.0	ND	ND	ND	ND

a- Cells were grown in Kp medium at 37°C

b- 1% sugar (final concentration)

c- Not detected

Table 45. Solvent production by *K. pneumoniae* grown anaerobically or under finite air conditions on pentoses.[a]

C Source[b]	Growth Condition		O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
						Ethanol	Acetic Acid	Butanediol	Total
L-arabinose	N_2	1 d	1.09	5.50	100.0	1.42	0.22	2.37	4.01
		2 d	0.92	5.30	100.0	1.34	0.30	2.50	4.14
		5 d	1.06	5.42	100.0	1.59	0.37	2.53	4.49
	FA[c]	1 d	2.02	6.15	100.0	0.70	0.93	1.80	3.43
		2 d	2.06	6.00	100.0	0.84	1.24	2.26	4.34
		5 d	1.95	6.07	100.0	0.73	1.15	1.70	3.58
D-xylose	N_2	1 d	0.84	5.45	57.5	1.06	0.93	0.41	2.40
		2 d	0.60	5.20	60.2	0.96	0.76	0.46	2.18
		5 d	0.81	5.26	62.8	1.13	0.91	0.48	2.52
	FA	1 d	1.99	5.50	100.0	0.83	1.02	2.33	4.18
		2 d	2.08	5.40	100.0	1.03	1.05	2.69	4.77
		5 d	2.06	5.40	100.0	0.93	0.90	2.30	4.13

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm)
b- 1% solution (final concentration)
c- finite air conditions

Table 46. Solvent production by *K. pneumoniae* grown anaerobically or under finite air conditions on hexoses.[a]

C Source[b]	Growth Condition		O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
						Ethanol	Acetic Acid	Butanediol	Total
D-glucose	N_2	1 d	1.41	5.40	100.0	1.75	0.29	2.72	4.76
		2 d	1.29	5.25	100.0	1.77	0.14	3.43	5.34
		5 d	1.40	5.38	100.0	1.71	0.39	2.40	4.50
	FA[c]	1 d	2.07	6.05	100.0	0.78	0.87	2.07	3.72
		2 d	2.08	6.00	100.0	0.74	0.96	2.14	3.84
		5 d	2.06	5.98	100.0	0.73	1.07	1.86	3.66
D-galactose	N_2	1 d	1.15	5.60	88.5	1.51	0.34	1.81	3.66
		2 d	1.03	5.30	100.0	1.36	0.26	2.26	3.88
		5 d	1.07	5.50	100.0	1.62	0.39	2.23	4.24
	FA	1 d	2.01	6.20	100.0	0.77	0.73	1.40	2.90
		2 d	2.03	6.00	100.0	0.65	0.93	1.44	3.02
		5 d	1.97	6.08	100.0	0.71	0.96	1.40	3.07
D-mannose	N_2	1 d	1.27	5.30	100.0	1.60	0.44	3.00	5.04
		2 d	1.13	5.25	100.0	1.49	0.28	2.63	4.40
		5 d	1.31	5.26	100.0	1.50	0.43	2.09	4.02
	FA	1 d	2.05	6.00	100.0	0.81	0.68	1.65	3.14
		2 d	2.10	5.90	100.0	0.98	1.51	2.48	4.97
		5 d	2.05	5.92	100.0	0.74	1.04	1.41	3.19

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm)

b- 1% solution (final concentration)

c- finite air conditions

Table 47. Solvent production by *K. pneumoniae* grown anaerobically or under finite air conditions on cellobiose.[a]

Growth Condition	Time of Incubation (days)	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
N_2	1	1.11	5.30	72.2	1.30	0.50	1.10	2.90
	2	0.88	5.20	74.8	1.19	0.54	0.95	2.68
	5	1.15	5.24	78.8	1.34	1.10	1.15	3.59
FA[b]	1	2.05	6.05	100.0	0.76	0.71	1.41	2.88
	2	2.01	5.90	100.0	0.86	1.03	1.87	3.76
	5	2.05	5.87	100.0	0.89	0.78	1.77	3.34

a- Cultures were grown in Kp medium (1% D-cellobiose) and incubated at 37°C with shaking (150 rpm)

b- Finite air

Table 48. Solvent production by *K. pneumoniae* grown on cellulosic substrates under finite air conditions (5% Inoculum used).[a]

Substrates[b]	O.D. (540 nm)	Final pH	Solvents (g/L)			
			Ethanol	Acetic Acid	Butanediol	Total
CF11	n/a[c]	7.01	0.09	0.25	ND[d]	0.34
CMC	0.227	7.14	0.08	0.23	ND	0.31
Solka Floc	n/a	7.13	0.08	0.13	ND	0.21
Kleenex	n/a	7.13	0.06	0.20	ND	0.26
Avicell	n/a	7.14	0.08	0.14	ND	0.22
Salicin	1.007	5.79	0.09	0.15	0.02	0.26
Xylan	0.543	7.01	0.47	1.62	ND	2.09
D-arabinose	1.698	5.56	0.64	0.81	ND	1.45
D-glucose	1.114	5.28	1.60	0.27	2.89	4.76

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm) for 14 days
b- All substrates at 1% final concentration
c- Not applicable due to presence of precipitate
d- Not detected

Table 49. Solvent production by *K. pneumoniae* grown on cellulosic substrates with large inoculum size (30%).[a]

Substrates[b]	O.D. (540 nm)	Final pH	Solvents (g/L)			
			Ethanol	Acetic Acid	Butanediol	Total
CF11	n/a[c]	6.80	0.22	0.43	ND[d]	0.65
CMC	0.783	6.80	0.35	0.50	ND	0.85
Solka Floc	n/a	6.85	0.31	0.35	ND	0.66
Kleenex	n/a	6.82	0.32	0.39	ND	0.71
Avicell	n/a	6.83	0.37	0.56	ND	0.93
Salicin	1.126	6.01	0.20	0.38	ND	0.58
Xylan	0.857	6.81	0.57	1.03	0.44	2.04
D-arabinose	1.608	5.60	0.65	1.24	ND	1.89

a- Cells were grown in Kp medium at 37°C with shaking (150 rpm) for 14 days
b- All substrates at 1% final concentration
c- Not applicable due to presence of precipitate
d- Not detected

Table 50. Solvent production by *K. pneumoniae* grown on wood hydrolyzate.[a]

Substrates	Incubation Time (days)	%C utilized[c]	Solvents (g/L)			Total
			Ethanol	Acetic Acid	Butanediol	
Xylan (Sigma)	2	92.0	1.77	0.99	4.81	7.57
	5	92.0	0.98	1.55	4.76	7.29
	7[b]	80.0	1.71	0.99	4.71	7.41
SEA (Solvent extracted aspen)	2	100.0	0.82	3.32	1.27	5.41
	5	100.0	0.93	3.73	1.46	6.12
	7	43.8	0.80	2.70	0.93	4.43
SEA	2	91.3	1.00	2.12	1.67	4.79
	5	91.3	0.96	2.99	1.75	5.70
	7	100.0	0.88	1.83	1.36	4.07
SEW-WS1 (undiluted) (Steam-exploded wood, water solubles)	2	76.8	2.18	5.72	0.19	8.09
	5	67.9	0.20	2.69	0.23	3.12
	7	71.5	0.13	2.00	0.16	2.29
SEW-WS1	2	32.0	0.48	3.31	0.31	4.10
	5	100.0	0.53	3.83	0.18	4.54
	7	47.4	0.55	3.41	0.27	4.23

a- Cultures were grown on wood samples prepared as described in Materials and Methods. Incubation was carried out at 37°C with shaking under finite air conditions.

b- Media used in experiment for 7 days incubation time were autoclaved with slow exhaust, resulting in browning of the solution.

c- Utilization of monosaccharides as monitoreed by HPLC

Table 51. Effect of $CaCO_3$ on solvent production of _K_. _pneumoniae_.[a]

C Source[b]	$CaCO_3$ Conc. (%)	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			Total
					Ethanol	Acetic Acid	Butanediol	
D-glucose	0.0	4.57	4.99	100.0	1.17	0.86	5.35	7.38
	0.2	5.06	6.23	98.7	1.40	1.77	4.45	7.62
	0.5	5.28	6.54	100.0	1.43	2.35	4.58	8.36
	1.0	4.81	6.72	100.0	1.55	2.22	4.78	8.55
D-xylose	0.0	4.20	4.87	92.5	1.56	1.23	4.07	7.86
	0.2	5.03	6.07	100.0	1.38	1.78	3.92	7.08
	0.5	5.17	6.28	100.0	1.63	2.70	3.31	7.64
	1.0	5.03	6.56	100.0	1.81	2.94	3.10	7.85

a- Cultures were grown in Kp medium supplemented with $CaCO_3$ and incubated at 37°C with shaking (120 rpm) under finite air conditions for 2 days

b- 2% sugar (final concentration)

Table 52. Effect of MES on solvent production of *K. pneumoniae*.[a]

Medium	C Source[b]	Growth Condition	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
						Ethanol	Acetic Acid	Butanediol	Total
Kp	D-glucose	Finite air	4.57	4.99	100.0	1.17	0.86	5.35	7.38
		N_2	1.76	5.10	72.4	2.03	1.13	3.48	6.64
	D-xylose	Finite air	4.20	4.87	92.5	1.56	1.23	4.07	7.86
		N_2	0.81	5.12	38.5	1.10	1.53	0.51	3.14
Kp+MES (1.0%)	D-glucose	Finite air	4.46	5.52	97.9	1.47	0.67	6.82	8.96
		N_2	1.37	5.28	76.5	2.30	0.81	4.07	7.18
	D-xylose	Finite air	3.90	5.47	91.0	1.70	0.88	6.12	8.70
		N_2	0.66	5.60	55.0	1.42	0.97	1.64	4.03

a- Cultures were incubated at 37°C with shaking (120 rpm) for 2 days

b- 2% sugar (final concentration)

Table 53. Effect of media modification on solvent production of K. pneumoniae.[a]

C Source[b]	Modification	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
D-glucose	No	4.46	5.52	97.9	1.47	0.67	6.82	8.96
	-EDTA	5.18	5.67	97.4	1.60	1.17	4.18	7.95
	-EDTA, +citric	4.82	5.75	100.0	1.63	1.42	5.51	8.56
	-K_2HPO_4	4.66	5.05	88.8	1.41	0.68	7.16	9.25
	-K_2HPO_4,-EDTA, +citric	4.10	5.47	100.0	1.28	0.87	4.53	6.68
D-xylose	No	3.90	5.47	91.0	1.70	0.88	6.12	8.70
	-EDTA	4.91	5.60	100.0	1.18	1.26	5.42	8.86
	-EDTA, +citric	4.73	5.80	100.0	1.88	1.87	4.63	7.38
	-K_2HPO_4	3.52	5.15	82.8	1.41	1.02	4.30	6.73
	-K_2HPO_4, -EDTA, +citric	4.18	5.38	100.0	1.65	1.30	4.65	7.60

a- Cultures were grown in Kp + MES (pH 6.5) at 37°C with shaking (120 rpm) under finite air conditions for 2 days

b- 2% sugar, final concentration

Table 54. Effect of initial pH on solvent production of *K. pneumoniae* grown under finite air conditions.[a]

C Source[b]	Initial pH	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
D-glucose	7.0	n.d.[c]	5.76	98.0	1.51	1.24	5.62	8.37
	6.5	n.d.	5.48	98.0	1.37	1.14	6.40	8.91
	6.0	n.d.	5.01	100.0	1.15	1.02	5.68	7.85
	5.5	n.d.	4.22	89.0	0.49	0.97	5.06	6.52
D-xylose	7.0	2.015	5.64	92.3	1.57	1.37	5.14	8.08
	6.5	1.950	5.47	89.3	1.45	1.13	5.48	8.06
	6.0	2.011	5.08	86.0	1.14	1.03	5.11	7.28
	5.5	2.050	4.31	64.4	0.59	0.85	4.26	5.70

a- Cultures were grown in Kp + MES (1.0%) and incubated at 37°C with shaking (120 rpm) for 1 day. Inoculum used was on a 1 day aerobic culture grown on glucose

b- 2% sugar (final concentration)

c- Not determined

Table 55. Effect of initial pH on solvent production of *K. pneumoniae* grown under finite air conditions, using an anaerobic culture as inoculum.[a]

C Source[b]	Initial pH	Final pH	% C utilized	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
D-glucose	7.0	5.77	98.5	1.32	0.80	5.06	7.18
	6.5	5.42	100.0	1.60	0.60	7.03	9.23
	6.0	4.94	89.8	1.17	0.75	6.05	7.97
	5.5	4.25	96.1	1.67	1.30	5.62	8.59
D-xylose	7.0	5.48	70.4	0.42	0.77	4.62	5.81
	6.5	5.41	92.4	1.48	0.81	5.40	7.69
	6.0	5.07	83.4	1.11	0.80	4.99	6.90
	5.5	4.36	67.0	0.55	0.82	4.59	5.96

a- Cultures were grown in Kp + Mes (1.0%) and incubated at 37°C for 4 days with shaking (120 rpm).
b- 2% sugar (final concentration)

Table 56. Effect of initial pH on solvent production of K. pneumoniae grown under finite air conditions in medium with high levels of trace elements.[a]

C Source[b]	Initial pH	Incubation Time (days)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
D-glucose	7.0	1	5.87	1.43	0.66	5.54	7.63
		4	5.86	1.42	0.78	5.82	8.02
	6.5	1	5.51	1.34	0.77	6.85	8.96
		4	5.49	1.37	0.81	7.46	9.64
	6.0	1	4.92	1.09	1.01	5.91	8.01
		4	4.92	0.95	0.69	6.15	7.79
	5.5	1	4.09	0.47	1.03	5.56	7.06
		4	4.14	0.44	0.71	5.94	7.09
D-xylose	7.0	1	5.68	1.76	1.47	4.97	8.20
		4	5.68	1.42	1.17	5.61	8.20
	6.5	1	5.45	1.49	0.95	5.28	7.72
		4	5.45	1.51	1.14	5.77	8.42
	6.0	1	4.98	0.98	1.16	4.74	6.88
		4	4.94	1.03	0.67	5.19	6.89
	5.5	1	4.32	0.69	1.14	3.76	5.59
		4	4.16	0.56	0.72	3.91	5.19

a- Cultures were grown in Kp + MES (1.0%) at 37°C with shaking (120 rpm). Trace elements were 10X more concentrated than the original medium

b- 2% sugar (final concentration)

Table 57. Effect of initial pH on solvent production of *K. pneumoniae* grown anaerobically (N_2).[a]

C Source[b]	Initial pH	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
D-glucose	7.0	1.149	5.56	1.76	0.47	2.65	4.88
	6.5	0.869	5.41	1.44	0.53	2.26	4.23
	6.0	0.484	5.13	0.95	0.33	1.57	2.85
	5.5	0.314	4.92	0.60	0.28	0.93	1.81
D-xylose	7.0	0.774	6.10	1.01	0.98	0.21	2.20
	6.5	0.328	6.08	0.56	0.69	0.10	1.35
	6.0	0.198	5.62	0.45	0.41	0.17	1.03

a- Cultures were grown in Kp + MES (1.0%) at 37°C with shaking (120 rpm) for 1 day. Inoculum used was 1 day aerobic culture grown on glucose.

b- 2% sugar (final concentration)

Table 58. Effect of initial pH on solvent production of *K. pneumoniae* grown anaerobically, using an anaerobic culture as inoculum.[a]

C Source[b]	Initial pH	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
D-glucose	7.0	1.16	5.71	1.37	0.81	1.41	3.59
	6.5	0.60	5.72	0.81	0.64	0.75	2.20
	6.0	0.25	5.64	0.29	0.38	0.13	0.80
	5.5	0.12	5.38	0.13	0.23	0.06	0.42
D-xylose	7.0	0.47	6.59	0.41	0.53	0.03	0.97
	6.5	0.27	6.34	0.30	0.35	0.03	0.68
	6.0	0.11	5.97	0.16	0.19	0.03	0.38
	5.5	0.07	5.49	0.15	0.15	0.01	0.31

a- Cultures were grown in Kp + MES (1.0%) at 37°C with shaking (120 rpm) for 1 day under N_2

b- 2% sugar (final concentration)

Table 59. Effect of initial pH on solvent production of *K. pneumoniae* grown anaerobically, using an anaerobic culture as inoculum.[a]

C Source[b]	Initial pH	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
D-glucose	7.0	1.16	5.43	96.1	2.66	0.77	5.80	9.23
	6.5	0.76	5.34	71.2	0.92	0.85	3.19	4.96
	6.0	0.54	5.04	53.0	1.21	0.70	2.62	4.53
	5.5	0.22	4.77	30.8	0.48	0.50	0.99	1.97
D-xylose	7.0	0.89	5.42	68.5	1.58	1.03	1.94	4.55
	6.5	0.67	5.46	56.0	1.60	0.58	3.05	5.23
	6.0	0.40	5.23	36.0	0.95	0.56	1.44	2.95
	5.5	0.16	5.28	14.5	0.22	0.44	0.06	0.72

a- Cultures were grown in Kp + MES (1%) and incubated under N_2 at 37°C with shaking (120 rpm) for 4 days

b- 2% sugar (final concentration)

Table 60. Solvent production of K. pneumoniae at initial pH of 7.5.[a]

C Source[b]	Growth Condition	Incubation Time (days)	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
						Ethanol	Acetic Acid	Butanediol	Total
D-glucose	Finite Air	1	4.97	5.90	100.0	1.59	1.28	5.21	8.08
		4	4.82	5.88	100.0	1.51	1.02	5.47	8.00
	N_2	2	1.98	5.51	100.0	2.78	1.22	4.23	8.23
		4	2.18	5.56	100.0	2.82	0.95	4.79	8.66
D-xylose	Finite Air	1	4.26	5.61	100.0	1.80	1.53	4.75	8.08
		4	4.52	5.63	100.0	1.59	1.12	5.22	7.93
	N_2	2	2.07	5.72	57.5	2.51	1.42	2.88	6.81
		4	2.03	5.59	89.0	2.58	1.12	4.17	7.87

a- Cultures were grown in Kp + MES (1.0%) at 37°C with shaking (120 rpm)
b- 2% sugar (final concentration)

Table 61. Effect of growth temperature on solvent production by *K. pneumoniae* grown anaerobically.[a]

C Source[b]	Temp (°C)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
D-glucose	37	0.70	5.38	1.23	0.50	2.78	4.51
	30	1.14	5.39	1.53	0.40	2.70	4.63
	28	1.14	5.46	1.37	0.61	2.51	4.49
D-xylose	37	0.42	5.86	0.74	0.89	0.16	1.79
	30	0.60	5.90	0.68	0.78	0.18	1.64
	28	0.42	5.96	0.55	0.68	0.15	1.38

a- Cultures were grown in Kp + MES (1.0%), pH 6.5 and incubated for 1 day with shaking (120 rpm) under N_2. Inoculum used was an aerobic culture (1 day old) grown on glucose

b- 2% sugar (final concentration)

Table 62. Effect of growth temperature on solvent production by *K. pneumoniae* grown anaerobically using an anaerobic culture as inoculum.[a]

C Source[b]	Temp (°C)	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L)			
					Ethanol	Acetic Acid	Butanediol	Total
D-glucose	37	0.78	5.27	61.8	1.73	0.46	3.68	5.87
	30	1.09	5.09	77.4	2.03	0.24	4.75	7.02
	28	1.09	5.08	70.7	1.93	0.37	4.89	7.19
D-xylose	37	0.46	5.31	79.2	1.28	0.38	2.01	3.67
	30	0.74	5.39	71.9	2.31	0.14	4.98	7.43
	28	0.76	5.43	74.8	2.12	0.38	4.76	7.26

a- Cultures were grown in Kp + MES (1%), pH 6.5 and incubated for 5 days with shaking (120 rpm). Inoculum was an anaerobic culture grown on glucose.

b- 2% sugar (final concentration)

Table 63. Effect of growth temperature on solvent production of *K. pneumoniae* grown on D-glucose under various conditions.[a]

Growth Conditions	Initial pH	Temp (°C)	Incubation Time (days)	O.D. (540 nm)	Final pH	% C Utilized	Solvents (g/L)			
							Ethanol	Acetic Acid	Butanediol	Total
Finite Air	6.5	30	2	4.15	5.62	97.4	1.70	0.22	7.98	9.90
			5	4.01	5.75	100.0	1.53	0.65	7.47	9.65
		37	2	4.31	5.50	98.1	1.55	0.67	6.62	8.84
			5	4.45	5.58	100.0	1.35	0.58	6.91	8.84
	7.0	30	2	4.59	5.87	100.0	1.68	0.98	6.06	8.72
			5	4.24	5.90	100.0	1.67	0.95	6.13	8.75
		37	2	4.78	5.77	100.0	1.61	0.99	5.78	8.38
			5	4.67	5.81	100.0	1.41	0.83	5.94	8.18
N_2	6.5	30	2	1.81	5.26	85.5	2.50	0.57	3.94	7.01
			5	1.89	5.27	92.0	2.66	0.51	4.45	7.62
		37	2	1.41	5.32	76.7	2.38	0.72	3.19	6.29
			5	1.37	5.42	86.5	2.11	0.38	3.41	5.90
	7.0	30	2	2.26	5.50	100.0	2.96	0.92	6.30	10.28
			5	2.31	5.53	100.0	2.92	0.54	6.49	9.95
		37	2	1.95	5.48	100.0	3.12	0.84	5.17	9.13
			5	1.75	5.50	100.0	2.65	0.31	6.08	9.04

a- Cultures were grown in Kp + MES (1.0%) with 2% D-glucose and incubated with shaking (120 rpm)

Table 64. Effect of growth temperatures on solvent production of _K_. _pneumoniae_ grown on D-xylose under various conditions.[a]

Growth Conditions	Initial pH	Temp (°C)	Incubation Time (days)	O.D. (540 nm)	Final pH	% C Utilized	Solvents (g/L)			
							Ethanol	Acetic Acid	Butanediol	Total
Finite Air	6.5	30	2	3.71	5.38	92.3	1.71	0.84	6.01	8.66
			5	3.75	5.44	94.7	1.72	0.73	6.60	9.05
		37	2	3.71	5.40	90.8	1.54	0.95	5.85	8.34
			5	3.67	5.45	91.6	1.48	0.57	6.23	8.28
	7.0	30	2	3.97	5.56	97.0	1.85	1.28	4.22	7.35
			5	3.78	5.58	97.5	2.10	0.72	6.05	8.77
		37	2	4.15	5.53	100.0	1.80	1.22	3.89	6.91
			5	4.41	5.57	100.0	1.53	0.97	6.07	8.57
N_2	6.5	30	2	1.22	5.66	42.5	1.25	0.98	1.94	4.17
			5	1.20	5.41	73.8	2.48	0.47	5.61	8.56
		37	2	0.97	5.59	37.4	1.43	0.91	1.58	3.92
			5	0.86	5.50	56.5	1.88	0.34	4.28	6.50
	7.0	30	2	1.68	5.64	50.2	1.81	1.43	1.57	4.81
			5	1.70	5.44	75.5	2.37	0.91	4.45	7.73
		37	2	1.67	5.58	68.0	2.18	1.26	1.36	4.80
			5	1.32	5.48	72.0	2.15	0.81	3.65	6.61

a- Cultures were grown in Kp + MES (1.0%) with 2% D-xylose and incubated with shaking (120 rpm).

Table 65. Solvent production by *K. pneumoniae* grown on sugars present in wood hemicellulose.[a]

C Source[b]	O.D. (540 nm)	Final pH	% C utilized	Solvents (g/L) Ethanol	Acetic Acid	Butanediol	Total
D-xylose	3.73	5.30	94.0	1.71	1.15	6.19	9.05
L-arabinose	3.57	5.63	100.0	1.54	1.18	6.05	8.77
D-glucose	4.21	5.60	100.0	1.70	0.32	7.26	9.28
D-galactose	3.41	5.63	96.0	1.45	1.01	5.65	8.11
D-mannose	3.60	5.58	100.0	1.56	0.97	5.76	8.29
D-cellobiose	3.67	5.33	100.0	1.98	1.08	5.94	9.00

a- Cultures were grown in Kp + MES (1.0%, initial pH 6.5) under finite air conditions at 30°C with shaking 120 rpm) for 2 days

b- Initial sugar concentration 2%

Table 66. Solvent production by *K. pneumoniae* grown on uronic acids and end-products. [a]

C Source[b]	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
Glucuronic acid	3	2.92	5.73	0.25	3.34	0.35	3.94
Galacturonic acid	3	2.96	5.76	0.23	3.10	0.46	3.79
Lactic acid	3	1.65	6.55	0.16	0.81	0.04	1.01
	14	0.94	6.62	0.21	0.52	0.05	0.78
Acetic acid	3	0.21	6.51	0.18	5.53	0.02	5.73
	14	0.90	6.83	0.20	3.56	0.10	3.86

a- Cultures were grown in Kp + MES (1.0%, initial pH 6.5) under finite air condition at 30°C with shaking (120 rpm)

b- All substrates at 2% final concentration

Table 67. Effect of initial sugar concentration on solvent production of K. pneumoniae grown on D-glucose.[a]

D-glucose Conc. %	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
1.0	1	1.82	5.84	0.91	0.42	3.09	4.42
	2	1.84	5.84	1.03	0.82	2.40	4.25
2.0	1	1.88	5.58	1.63	0.08	6.42	8.13
	2	1.96	5.56	1.72	0.40	5.07	7.19
3.0	1	1.89	5.59	1.67	0.20	6.71	8.58
	2	1.97	5.55	1.75	0.88	5.30	7.93
	5	2.00	5.50	1.74	0.96	5.32	8.02
	8	n.d.[b]	n.d.	1.68	0.85	5.22	7.75
5.0	1	1.87	5.58	1.52	0.17	7.02	8.71
	2	1.98	5.55	1.85	1.03	5.39	8.27
	5	1.99	5.54	1.75	1.06	5.36	8.17
	8	n.d.	n.d.	1.70	0.81	5.48	7.99
8.0	1	1.79	5.61	1.39	0.29	4.54	6.22
	2	1.91	5.54	1.90	1.30	6.65	9.85
	5	1.93	5.56	1.94	1.82	5.31	9.07
	8	n.d.	n.d.	1.80	1.10	5.59	8.49
10.0	1	1.68	5.73	1.17	0.33	3.45	4.95
	2	1.83	5.61	1.92	1.28	6.40	9.60
	5	1.85	5.58	1.88	1.21	4.92	8.01
	8	n.d.	n.d.	1.76	1.06	5.34	8.16

---

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30^{C} with shaking (120 rpm), using 1 day aerobic culture as inoculum (2%, ^{v}/v)

b- Not determined

Table 68. Effect of initial sugar concentration on solvent production of *K. pneumoniae* grown on D-xylose.[a]

D-xylose Conc. %	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
1.0	1	1.66	5.72	1.04	0.63	1.65	3.32
	2	1.71	5.49	1.28	1.75	2.34	5.47
2.0	1	1.75	5.67	1.26	0.25	3.11	4.62
	2	1.83	5.52	1.92	1.27	5.17	8.36
3.0	1	1.74	5.68	1.17	0.38	2.76	4.31
	2	1.84	5.51	1.87	1.22	4.78	7.87
	5	1.87	5.43	1.89	1.22	3.87	6.98
	8	n.d.[b]	n.d.	1.78	0.87	3.17	5.82
5.0	1	1.68	5.71	0.96	0.61	1.99	3.56
	2	1.81	5.52	1.84	1.19	4.96	7.99
	5	1.86	5.45	1.85	1.27	3.89	7.01
	8	n.d.	n.d.	1.77	1.02	3.19	5.98
8.0	1	0.51	6.41	0.23	0.24	0.05	0.52
	2	1.74	5.62	1.24	1.43	3.21	5.88
	5	1.82	5.42	1.48	1.07	4.47	7.02
	8	n.d.	n.d.	1.51	0.69	4.22	6.42
10.0	1	0.11	6.49	0.22	0.02	0.03	0.27
	2	1.47	5.72	0.72	1.44	0.67	2.83
	5	1.70	5.32	1.40	1.01	3.83	6.24
	8	n.d.	n.d.	1.56	0.48	3.19	5.23

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm), using 1 day aerobic culture as inoculum (2%, $^{v}/v$)

b- Not determined

Table 69. Effect of inoculum size on solvent production of *K. pneumoniae* grown on various D-glucose concentrations.[a]

Glucose Conc. %	Inoculum Size %	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L) Ethanol	Acetic Acid	Butanediol	Total
2	5	1	1.88	5.58	1.57	0.16	6.99	8.72
		2	1.95	5.50	1.67	0.63	5.12	7.42
	10	1	1.84	5.60	1.65	0.19	6.79	8.63
		2	1.91	5.60	1.72	0.79	5.09	7.60
5	5	1	1.85	5.57	1.44	0.22	6.45	8.11
		2	1.93	5.57	1.54	1.18	5.80	8.52
		5	1.96	5.54	1.74	1.18	5.81	8.73
		8	n.d.[b]	n.d.	1.60	0.85	5.56	8.01
	10	1	1.85	5.61	1.68	0.17	6.09	7.94
		2	1.92	5.56	1.72	1.13	5.82	8.67
		5	1.94	5.50	1.79	1.14	5.43	8.36
		8	n.d.	n.d.	1.69	1.00	5.43	8.12
10	5	1	1.73	5.72	1.28	0.28	4.50	6.06
		2	1.84	5.59	1.73	1.44	6.29	9.46
		5	1.84	5.59	1.86	1.19	5.37	8.42
		8	n.d.	n.d.	1.73	1.01	5.75	8.49
	10	1	1.74	5.65	1.55	0.21	5.46	7.22
		2	1.81	5.61	1.72	1.52	6.32	9.56
		5	1.83	5.60	1.83	1.20	5.86	8.89
		8	n.d.	n.d.	1.80	0.95	5.72	8.47

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm)
b- Not determined

Table 70. Effect of inoculum size on solvent production of K. pneumoniae grown on various D-xylose concentrations.[a]

Xylose Conc. %	Inoculum Size %	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L) Ethanol	Acetic Acid	Butanediol	Total
2	5	1	1.77	5.65	1.31	0.60	3.35	5.26
		2	1.84	5.52	1.99	1.30	5.06	8.35
	10	1	1.73	5.65	1.30	0.64	3.47	5.41
		2	1.79	5.52	1.99	1.29	4.83	8.11
5	5	1	1.72	5.72	1.14	0.27	2.71	4.12
		2	1.82	5.52	1.60	1.22	4.62	7.44
		5	1.84	5.39	1.92	1.22	3.96	7.10
		8	n.d.[b]	n.d.	1.76	1.12	2.87	5.75
	10	1	1.76	5.62	1.34	0.37	3.35	5.06
		2	1.84	5.51	1.59	1.36	4.25	7.20
		5	1.88	5.37	1.92	1.21	3.81	6.94
		8	n.d.	n.d.	1.85	1.12	3.02	5.99
10	5	1	0.35	6.39	0.25	0.26	0.06	0.57
		2	1.66	5.66	1.00	1.14	2.10	4.24
		5	1.74	5.46	1.48	0.90	3.98	6.36
		8	n.d.	n.d.	1.61	0.39	3.21	5.21
	10	1	1.04	6.12	0.40	0.53	0.24	1.17
		2	1.71	5.61	1.35	0.88	3.82	6.05
		5	1.74	5.49	1.60	0.96	4.37	6.93
		8	n.d.	n.d.	1.63	0.35	3.56	5.54

---

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition with shaking (120 rpm) at 30°C

b- Not determined

Table 71. Effect of sugar concentration on solvent on production of *K. pneumoniae* upon prolonged incubation.[a]

C source	Sugar Conc. (%)	Inoculum Size (%)	Solvents (g/L)	
			Ethanol	Butanediol
D-glucose	1	2	1.00	3.03
	2	2	1.73	6.19
	2	5	1.56	6.83
	2	10	1.33	5.65
	3	2	1.41	6.55
	5	2	1.56	7.42
	5	5	1.44	7.41
	5	10	1.43	6.59
	8	2	1.42	6.26
	10	2	1.49	6.19
	10	5	1.40	6.19
	10	10	1.41	6.23
D-xylose	1	2	1.01	3.16
	2	2	1.34	4.58
	2	5	1.34	4.75
	2	10	1.40	5.02
	3	2	1.58	6.38
	5	2	1.38	5.34
	5	5	1.53	5.86
	5	10	1.54	5.82
	8	2	1.36	6.37
	10	2	1.24	5.26
	10	5	1.32	5.53
	10	10	1.44	5.75

a- Cultures were incubated in Kp + MES (1.0%, pH 6.5) at 30°C under finite air conditions with shaking (120 rpm) for 30 days

Table 72. Effect of a fed-batch system on solvent production by *K. pneumoniae* grown on D-glucose.[a]

Inoculum Size %	Incubation Time[b]	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
2	0	1.96	5.56	1.72	0.40	5.07	7.17
	1	n.d.[c]	n.d.	1.48	1.35	4.60	7.43
	3	1.93	5.48	1.60	0.49	4.23	6.32
	6	n.d.	n.d.	1.61	0.42	3.98	6.01
5	0	1.95	5.50	1.67	0.63	5.12	7.42
	1	n.d.	n.d.	1.58	1.35	4.85	7.78
	3	1.94	5.55	1.68	0.64	4.33	6.65
	6	n.d.	n.d.	1.60	0.53	4.67	6.80
10	0	1.91	5.60	1.72	0.79	5.09	7.60
	1	n.d.	n.d.	1.62	1.37	5.23	8.22
	3	1.89	5.50	1.68	0.83	4.51	7.02
	6	n.d.	n.d.	1.62	0.59	4.67	6.88

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm). Initial sugar concentration 2%. Additional 2% added at day 0

b- Cultures incubated for 2 days prior to addition of extra sugar at day 0

c- Not determined

Table 73. Effect of a fed-batch system on solvent production by *K*. *pneumoniae* grown on D-xylose.[a]

Inoculum Size %	Incubation Time[b]	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
2	0	1.83	5.52	1.92	1.27	5.17	8.36
	1	n.d.[c]	n.d.	1.56	1.47	4.01	7.04
	3	1.80	5.50	1.87	1.21	3.91	6.99
	6	n.d.	n.d.	1.77	0.84	2.95	5.56
5	0	1.84	5.52	1.99	1.30	5.06	8.35
	1	n.d.	n.d.	1.54	1.45	3.86	6.85
	3	1.80	5.52	1.82	1.21	3.56	6.59
	6	n.d.	n.d.	1.79	0.95	2.67	5.41
10	0	1.79	5.52	1.99	1.29	4.83	8.11
	1	n.d.	n.d.	1.54	1.23	3.94	6.71
	3	1.75	5.48	1.91	1.19	3.66	6.66
	6	n.d.	n.d.	1.75	0.85	2.98	5.58

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm). Initial sugar concentration 2%. Additional 2% added at day 0

b- Cultures incubated for 2 days prior to addition of extra sugar at day 0

c- Not determined

Table 74. Effect of nutrient supplement on solvent production of *K. pneumoniae* grown on a high concentration of D-glucose.[a]

Nutrient Supplement	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
NIL (control)	0	0.38	6.3	0.47	0.91	0.68	2.06
	1	4.38	5.5	1.27	1.39	6.78	9.44
	2	4.97	5.3	1.87	0.20	8.21	10.28
	4	5.25	5.4	1.82	0.75	8.09	10.66
1% yeast extract	1	5.18	5.6	2.15	0.86	11.10	14.11
	2	6.00	5.5	2.35	0.64	12.40	15.39
	4	6.40	5.5	2.43	0.35	12.27	15.05
1% urea	1	3.44	5.8	1.16	1.64	3.08	5.88
	2	4.24	5.6	2.52	0.75	10.49	13.76
	4	4.01	5.5	2.51	0.29	9.54	12.34
2 x $(NH_4)_2SO_4$	1	4.35	5.4	1.89	0.82	10.45	13.16
	2	4.95	5.4	1.56	0.60	6.91	9.07
	4	4.84	5.4	1.71	0.58	9.97	12.26
10 x trace metals	1	4.24	5.6	1.29	0.98	7.27	9.54
	2	4.83	5.3	1.78	0.48	9.64	11.90
	4	5.25	5.2	1.75	0.20	7.96	9.91

[a] Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm), using 4% D-glucose as substrate

Table 75. Effect of nutrient supplement on solvent production of *K. pneumoniae* grown on a high concentration of D-xylose.[a]

Nutrient Supplement	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)			
				Ethanol	Acetic Acid	Butanediol	Total
NIL (control)	0	0.43	6.4	0.35	0.39	0.13	0.87
	1	3.73	5.5	1.84	1.08	6.76	9.68
	2	3.96	5.4	1.84	0.59	7.55	9.98
	4	4.08	5.3	2.12	0.25	7.46	9.83
1% yeast extract	1	5.02	5.4	2.46	0.95	10.67	14.08
	2	5.96	5.4	2.59	0.42	11.30	14.31
	4	6.44	5.4	2.71	0.47	12.04	15.22
1% urea	1	3.90	5.8	1.76	1.31	5.69	8.76
	2	4.44	5.5	2.38	0.40	9.78	12.56
	4	3.39	5.5	2.67	0.35	10.34	13.36
2 x $(NH_4)_2SO_4$	1	3.90	5.5	1.68	0.83	6.86	9.37
	2	3.50	5.3	1.96	0.42	10.91	13.29
	4	n.d[b]	n.d.	n.d.	n.d.	n.d.	n.d.
10 x trace metals	1	3.91	5.3	1.74	0.78	6.98	9.50
	2	4.50	5.1	1.76	0.58	8.36	10.70
	4	4.77	5.1	1.64	0.50	6.44	8.58

a- Cultures were grown in Kp + MES (1.0%, pH 6.5, 4% D-xylose) under finite air conditions at 30°C with shaking (120 rpm)

b- Not determined

Table 76. Effect of end-products on solvent production of *K. pneumoniae* grown on D-glucose.[a]

End-product added[b]	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L) Ethanol	Solvents (g/L) Acetic Acid	Solvents (g/L) Butanediol
NIL (control)	0	0.2% C[c]	0.06	6.4	0.19	0.64	0.11
	1	1.6% C	1.24	6.3	0.40	1.05	1.18
	2	1.6% C	1.51	5.4	1.52	0.08	3.57
	5	0.4% YE	1.64	5.3	1.48	0.13	4.06
	6	NIL	1.60	5.6	1.27	0.21	3.52
0.5% Ethanol	0	0.2% C	0.07	6.5	6.51	0.14	0.02
	1	1.6% C	1.26	6.2	6.05	0.75	0.11
	2	1.6% C	1.48	5.5	6.65	0.33	2.86
	5	0.4% YE	1.53	5.5	7.39	0.07	5.21
	6	NIL	1.48	5.6	5.39	0.19	3.46
0.5% Acetic acid	0	0.2% C	0.06	6.5	0.15	3.26	0.0
	1	1.6% C	1.05	6.5	0.44	3.36	0.29
	2	1.6% C	1.94	6.2	1.88	2.07	7.87
	5	0.4% YE	1.90	6.0	2.74	0.83	13.83
	6	NIL	1.82	6.0	2.70	0.70	13.50
1.0% Butanediol	0	0.2% C	0.08	6.5	0.18	0.28	9.80
	1	1.6% C	1.16	6.1	0.35	0.96	7.99
	2	1.6% C	1.55	5.2	1.11	0.54	13.56
	5	0.4% YE	1.59	5.6	1.36	0.21	15.24
	6	NIL	1.51	5.6	1.15	0.19	12.20

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) at 30°C under finite air conditions with shaking (120 rpm)
b- End-products were added at 0 hour
c- C = Carbon source, YE = yeast extract

Table 77. Effect of end-products on solvent production of *K. pneumoniae* grown on D-xylose.[a]

End-product added[b]	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L) Ethanol	Acetic Acid	Butanediol
NIL (control)	0	0.2% C[c]	0.06	6.4	0.21	0.30	0.05
	1	1.6% C	1.12	6.3	0.30	0.66	0.24
	2	1.6% C	1.64	5.7	1.05	0.59	1.07
	5	0.4% YE	1.67	5.5	1.14	0.58	0.46
	6	NIL	1.55	5.3	0.90	0.60	0.98
0.5% Ethanol	0	0.2% C	0.07	6.5	6.29	0.09	0.00
	1	1.6% C	1.00	6.2	5.39	0.50	0.04
	2	1.6% C	1.32	5.2	6.42	0.63	0.86
	5	0.4% YE	1.39	5.2	6.42	0.61	1.24
	6	NIL	1.39	5.2	5.74	0.68	1.01
0.5% Acetic Acid	0	0.2% C	0.06	6.5	0.16	3.42	0.00
	1	1.6% C	1.17	6.3	0.42	3.41	0.00
	2	1.6% C	1.39	5.6	0.97	3.77	1.03
	5	0.4% YE	1.39	5.4	1.24	2.95	2.92
	6	NIL	1.31	5.4	0.91	2.47	2.11
1.0% Butanediol	0	0.2% C	0.05	6.5	0.18	0.12	10.70
	1	1.6% C	0.77	6.2	0.23	0.76	9.45
	2	1.6% C	1.17	5.3	0.58	0.76	9.97
	5	0.4% YE	1.18	5.1	0.66	0.56	10.98
	6	NIL	1.11	5.1	0.67	0.69	9.25

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) at 30°C under finite air conditions with shaking (120 rpm)

b- End-products were added at 0 hour

c- C = carbon source, YE = yeast extract

Table 78. Effect of ethanol added during fermentation on solvent production of K. pneumoniae grown on D-glucose.[a]

Time of addition	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L) Ethanol	Solvents (g/L) Acetic Acid	Solvents (g/L) Butanediol
8 h	8 (h)	0.2% C[b]	1.14	6.25	2.36	0.70	0.75
	1	1.6% C	1.15	6.30	1.68	0.87	1.10
	2	1.6% C	1.59	5.70	2.40	0.30	3.97
	5	0.4% YE	1.61	5.60	2.50	0.13	4.93
	6	NIL	1.53	5.60	2.24	0.26	4.00
1 d	0	0.2% C	0.06	6.40	0.19	0.64	0.11
	1	1.6% C	1.15	6.30	1.82	0.75	0.70
	2	1.6% C	1.58	5.40	2.55	0.28	4.49
	5	0.4% YE	1.61	5.40	2.54	0.12	5.04
	6	NIL	1.54	5.50	2.58	0.16	4.59

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). 0.2% ethanol was added at times indicated

b- C = carbon source, YE = yeast extract

Table 79. Effect of acetic acid added during fermentation on solvent production of *K. pneumoniae* grown on D-glucose.[a]

Time of addition	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L)		
					Ethanol	Acetic Acid	Butanediol
8 h	8 (h)	0.2% C[b]	1.13	5.15	0.49	0.61	0.52
	1	1.6% C	1.12	5.40	0.37	2.01	0.14
	2	1.6% C	0.92	5.40	0.51	2.15	0.90
	5	0.4% YE	0.84	5.60	0.60	1.72	1.72
	6	NIL	0.79	5.70	0.64	1.65	2.10
1 d	0	0.2% C	0.06	6.40	0.19	0.64	0.11
	1	1.6% C	1.12	5.50	0.41	2.11	0.51
	2	1.6% C	1.29	5.60	0.93	1.50	2.01
	5	0.4% YE	1.61	5.60	2.30	0.11	9.87
	6	NIL	1.54	5.60	2.13	0.17	9.09

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). 0.2% acetic acid was added at times indicated

b- C = carbon source, YE = yeast extract

Table 80. Effect of butanediol added during fermentation on solvent production of _K_. _pneumoniae_ grown on D-glucose.[a]

Time of addition	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L)		
					Ethanol	Acetic Acid	Butanediol
8 h	8 (h)	0.2% C[b]	1.11	6.20	0.31	0.70	8.95
	1	1.6% C	1.13	6.10	0.34	0.81	8.54
	2	1.6% C	1.60	5.40	1.16	0.26	14.30
	5	0.4% YE	1.63	5.50	1.29	0.22	15.19
	6	NIL	1.55	5.60	0.71	1.74	0.03
1 d	0	0.2% C	0.06	6.40	0.19	0.64	0.11
	1	1.6% C	1.12	6.20	0.25	0.27	6.00
	2	1.6% C	1.58	5.50	1.45	0.45	17.26
	5	0.4% YE	1.62	5.50	1.48	0.18	16.04
	6	NIL	1.56	5.50	1.28	0.23	14.53

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). 0.5% butanediol was added at times indicated

b- C = carbon source, YE = yeast extract

Table 81. Effect of ethanol added during fermentation on solvent production of *K. pneumoniae* grown on D-xylose.[a]

Time of addition	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L)		
					Ethanol	Acetic Acid	Butanediol
1 d	0	0.2% C[b]	0.08	6.40	0.21	0.30	0.05
	1	1.6% C	1.08	6.30	1.60	0.59	0.34
	2	1.6% C	1.46	5.20	3.14	0.30	4.22
	5	0.4% YE	1.52	5.20	1.98	0.69	0.90
	6	NIL	1.49	5.20	1.80	0.75	0.70
2 d	0	0.2% C	0.08	6.40	0.21	0.30	0.05
	1	1.6% C	1.12	6.30	0.30	0.66	0.24
	2	1.6% C	1.40	5.30	3.07	0.62	1.30
	5	0.4% YE	1.47	5.20	2.25	0.61	1.18
	6	NIL	1.42	5.10	3.04	0.42	3.53

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Ethanol (0.2%) was added at times indicated

b- C = carbon source, YE = yeast extract

Table 82. Effect of acetic acid added during fermentation on solvent production of *K. pneumoniae* grown on D-xylose.[a]

Time of addition	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L)		
					Ethanol	Acetic Acid	Butanediol
1 d	0	0.2% C[b]	0.08	6.40	0.21	0.30	0.05
	1	1.6% C	1.03	5.50	0.32	1.99	0.04
	2	1.6% C	0.90	5.20	0.52	1.83	0.09
	5	0.4% YE	0.83	5.20	0.70	1.85	0.07
	6	NIL	0.83	5.40	0.53	1.80	0.12
2 d	0	0.2% C	0.08	6.40	0.21	0.30	0.05
	1	1.6% C	1.12	6.30	0.30	0.66	0.24
	2	1.6% C	1.44	4.50	0.97	2.54	0.89
	5	0.4% YE	1.60	4.70	1.01	1.82	1.47
	6	NIL	1.53	4.80	0.83	1.73	1.31

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Acetic acid (0.2%) was added at times indicated

b- C = carbon source, YE = yeast extract

Table 83. Effect of butanediol added during fermentation on solvent production of *K. pneumoniae* grown on D-xylose.[a]

Time of addition	Incubation Time (days)	Medium Supplement	O.D. (540 nm)	Final pH	Solvents (g/L) Ethanol	Acetic Acid	Butanediol
1 d	0	0.2% C[b]	0.08	6.40	0.21	0.30	0.05
	1	1.6% C	1.08	6.20	0.30	0.58	11.55
	2	1.6% C	1.39	5.20	0.68	0.88	10.90
	5	0.4% YE	1.48	5.20	0.78	0.68	10.88
	6	NIL	1.43	5.10	0.75	0.76	9.03
2 d	0	0.2% C	0.08	6.40	0.21	0.30	0.05
	1	1.6% C	1.12	6.30	0.30	0.66	0.24
	2	1.6% C	1.44	5.20	0.95	0.67	16.94
	5	0.4% YE	1.49	5.10	0.88	0.56	11.85
	6	NIL	1.44	5.20	0.75	0.67	10.85

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Butanediol (0.5%) was added at times indicated

b- C = carbon source, YE = yeast extract

Table 84. Effect of end-products on solvent production of _K_. _pneumoniae_ grown on D-glucose.[a]

End-product added[b]	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
NIL (control)	0	0.20	6.40	0.31	1.36	0.10
	1	1.64	5.40	1.50	0.84	7.33
	2	1.70	5.50	1.00	0.69	4.63
	5	1.75	5.50	1.11	0.66	4.19
1.0% Ethanol	0	0.20	6.30	7.42	0.25	0.00
	1	1.42	5.50	7.08	0.54	4.02
	2	1.50	5.50	1.94	0.76	4.01
	5	1.58	5.50	7.62	0.48	5.46
1.0% Acetic acid	0	0.19	6.80	0.39	6.64	0.13
	1	0.22	8.20	0.53	6.39	0.56
	2	0.14	8.50	0.25	7.18	0.27
	5	0.14	8.30	0.39	7.82	0.47
2.0% Butanediol	0	0.19	6.20	0.32	0.99	19.50
	1	1.34	5.50	0.88	1.09	25.23
	2	1.39	5.30	1.00	1.46	22.75
	5	1.46	5.30	1.19	1.06	27.98

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) at 30°C under finite air condition with shaking (120 rpm) Initial glucose concentration at 2.0%

b- End-products were added at day 0

Table 85. Effect of end-products on solvent production of *K. pneumoniae* grown on D-xylose.[a]

End-product added[b]	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
NIL (control)	0	0.18	6.2	0.27	0.55	0.00
	1	1.56	5.5	1.04	0.97	3.29
	2	1.66	5.4	1.19	0.73	3.83
	5	1.73	5.5	1.43	0.59	4.72
1.0% Ethanol	0	0.20	6.3	6.95	0.19	0.08
	1	1.28	5.4	7.26	0.55	3.46
	2	1.31	5.4	7.68	0.51	3.88
	5	1.47	5.4	8.68	0.46	5.00
1.0% Acetic acid	0	0.16	6.5	0.36	7.82	0.07
	1	0.34	6.4	0.40	7.43	0.33
	2	0.27	6.4	0.37	7.71	0.21
	5	0.24	6.4	0.62	8.54	0.25
2.0% Butanediol	0	0.17	6.3	0.25	1.20	21.24
	1	1.18	5.6	0.69	1.24	25.64
	2	1.27	5.5	0.99	1.92	23.44
	5	1.37	5.4	1.25	1.20	27.06

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) at 30°C under finite air condition with shaking (120 rpm)
Initial D-xylose concentration was 2.0%

b- End-products were added at day 0

Table 86. Effect of ethanol on solvent production of *K. pneumoniae* grown on D-glucose.[a]

Time of Addition	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
6 h	6 (h)	0.93	6.0	4.68	0.84	1.69
	1	1.47	5.4	5.28	0.56	7.11
	2	1.53	5.3	4.91	1.54	6.29
	5	1.62	5.5	4.74	0.94	6.11
1 d	0	0.20	6.4	0.31	1.36	0.10
	1	1.37	5.6	1.20	1.40	7.60
	2	1.58	5.7	4.79	1.39	6.13
	5	1.66	5.4	5.31	0.87	6.73

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Ethanol (0.5% final concentration) was added at times indicated. Initial glucose concentration 2.0%.

Table 87. Effect of acetic acid on solvent production of *K. pneumoniae* grown on D-glucose.[a]

Time of Addition	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
6 h	6 (h)	0.82	6.0	0.36	0.70	2.94
	1	1.34	5.4	1.02	1.28	4.71
	2	1.60	5.6	2.12	0.75	8.26
	5	1.65	5.6	6.77	0.60	4.57
1 d	0	0.20	6.4	0.31	1.36	0.10
	1	1.51	5.4	0.75	1.60	3.09
	2	1.62	5.7	2.10	0.87	8.88
	5	1.67	5.6	1.97	0.61	9.83

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Initial glucose concentration 2.0%. Acetic acid (final concentration 0.5%) was added at times indicated.

Table 88. Effect of butanediol on solvent production of _K_. _pneumoniae_ grown on D-glucose.[a]

Time of Addition	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
6 h	6 (h)	0.86	5.8	0.36	1.02	35.12
	1	1.55	5.4	1.04	0.45	32.94
	2	1.49	5.3	1.14	0.79	32.40
	5	1.40	5.2	1.01	1.14	28.41
1 d	0	0.20	6.4	0.31	1.36	0.10
	1	1.34	5.5	0.83	0.83	28.22
	2	1.64	5.4	1.15	0.56	37.08
	5	1.55	5.3	1.27	0.92	35.09

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Initial glucose concentration 2.0%. Butanediol (final concentration 2.5%) was added at times indicated.

Table 89. Effect of ethanol on solvent production of *K. pneumoniae* grown on D-xylose.[a]

Time of Addition	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
1 d	0	0.18	6.20	0.27	0.55	0.00
	1	1.49	5.70	4.87	0.63	2.89
	2	1.47	5.40	5.14	0.73	4.08
	5	1.58	5.60	5.37	0.68	5.22
2 d	1	1.56	5.50	1.04	0.97	3.29
	2	1.48	5.50	5.06	1.09	4.72
	5	1.58	5.80	5.58	0.69	5.25

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Initial xylose concentration was 2%. Ethanol (final concentration 0.5%) was added at times indicated.

Table 90. Effect of acetic acid on solvent production of _K_. _pneumoniae_ grown on D-xylose.[a]

Time of Addition	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
1 d	0	0.18	6.20	0.27	0.55	0.00
	1	1.38	5.50	4.50	0.27	4.57
	2	1.49	5.60	1.52	1.28	5.15
	5	1.52	5.70	2.01	0.64	7.87
2 d	1	1.56	5.50	1.04	0.97	3.29
	2	1.51	5.60	1.13	1.78	4.26
	5	1.53	5.50	1.39	1.51	4.80

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Initial xylose concentration was 2.0%. Acetic Acid (final concentration 0.5%) was added at times indicated.

Table 91. Effect of butanediol on solvent production of *K. pneumoniae* grown on D-xylose.[a]

Time of Addition	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
1 d	0	0.18	6.20	0.27	0.55	0.00
	1	1.49	5.70	0.99	0.49	37.56
	2	1.37	5.30	1.29	1.44	28.14
	5	1.51	5.40	1.44	0.87	37.10
2 d	1	1.56	5.50	1.04	0.97	3.29
	2	1.49	5.30	1.36	1.37	31.67
	5	1.62	5.50	1.27	0.79	38.89

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air conditions at 30°C with shaking (120 rpm). Initial xylose concentration was 2.0%. Butanediol (final concentration 2.5%) was added at times indicated.

Table 92. Effect of various acetic acid concentrations on solvent production of *K. pneumoniae* grown on D-glucose.[a]

Acetic acid Conc. (%)	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
0.0	0	0.40	6.4	0.11	0.18	0.00
	1	5.16	5.5	1.43	0.09	8.74
	2	5.33	5.4	1.89	0.16	8.86
	5	5.27	5.3	1.29	0.57	6.70
0.1	1	5.81	5.6	2.76	0.27	16.34
	2	6.04	5.5	1.82	0.38	7.58
	5	5.81	5.6	2.48	0.79	12.63
0.3	1	6.48	5.9	3.38	1.13	19.35
	2	6.00	5.9	2.84	1.58	15.75
	5	4.83	6.0	2.58	2.23	14.20
0.5	1	6.29	6.1	3.14	1.71	19.61
	2	5.14	6.1	2.73	2.31	16.75
	5	3.82	6.0	2.49	3.01	13.93
1.0	1	1.49	6.3	0.23	7.21	2.63
	2	1.87	6.4	1.05	6.73	9.49
	5	2.83	6.2	2.50	5.94	15.88
2.0	1	0.50	6.5	0.15	12.00	0.75
	2	0.51	6.5	0.19	12.29	1.64
	5	0.50	6.3	0.28	13.57	2.61

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm). Initial D-glucose concentration was 4.0%

Table 93. Effect of various acetic acid concentrations on solvent production of *K. pneumoniae* grown on D-xylose.[a]

Acetic acid Conc. (%)	Incubation Time (days)	O.D. (540 nm)	Final pH	Solvents (g/L)		
				Ethanol	Acetic Acid	Butanediol
0.0	0	0.37	6.5	0.18	0.19	0.12
	1	3.93	5.4	1.60	0.34	7.29
	2	4.21	5.3	1.86	0.35	7.79
	5	4.26	5.3	1.78	0.68	6.24
0.1	1	4.80	5.6	2.06	0.96	10.61
	2	5.52	5.6	2.65	0.41	14.18
	5	4.88	5.5	2.58	0.14	12.10
0.3	1	5.42	5.8	2.00	2.03	10.43
	2	5.92	5.8	4.06	0.55	20.19
	5	5.37	5.8	3.39	1.31	15.23
0.5	1	5.02	6.1	1.69	2.98	9.18
	2	5.64	6.0	4.29	1.05	22.00
	5	5.16	5.9	3.62	2.33	18.67
1.0	1	0.71	6.4	0.20	7.41	0.62
	2	1.48	6.4	0.25	7.18	3.02
	5	2.62	6.1	1.52	6.70	9.59
2.0	1	0.25	6.5	0.17	12.10	0.11
	2	0.24	6.5	0.17	12.48	0.29
	5	0.26	6.4	0.23	14.06	0.41

a- Cultures were grown in Kp + MES (1.0%, pH 6.5) under finite air condition at 30°C with shaking (120 rpm). Initial D-xylose concentration was 4.0%

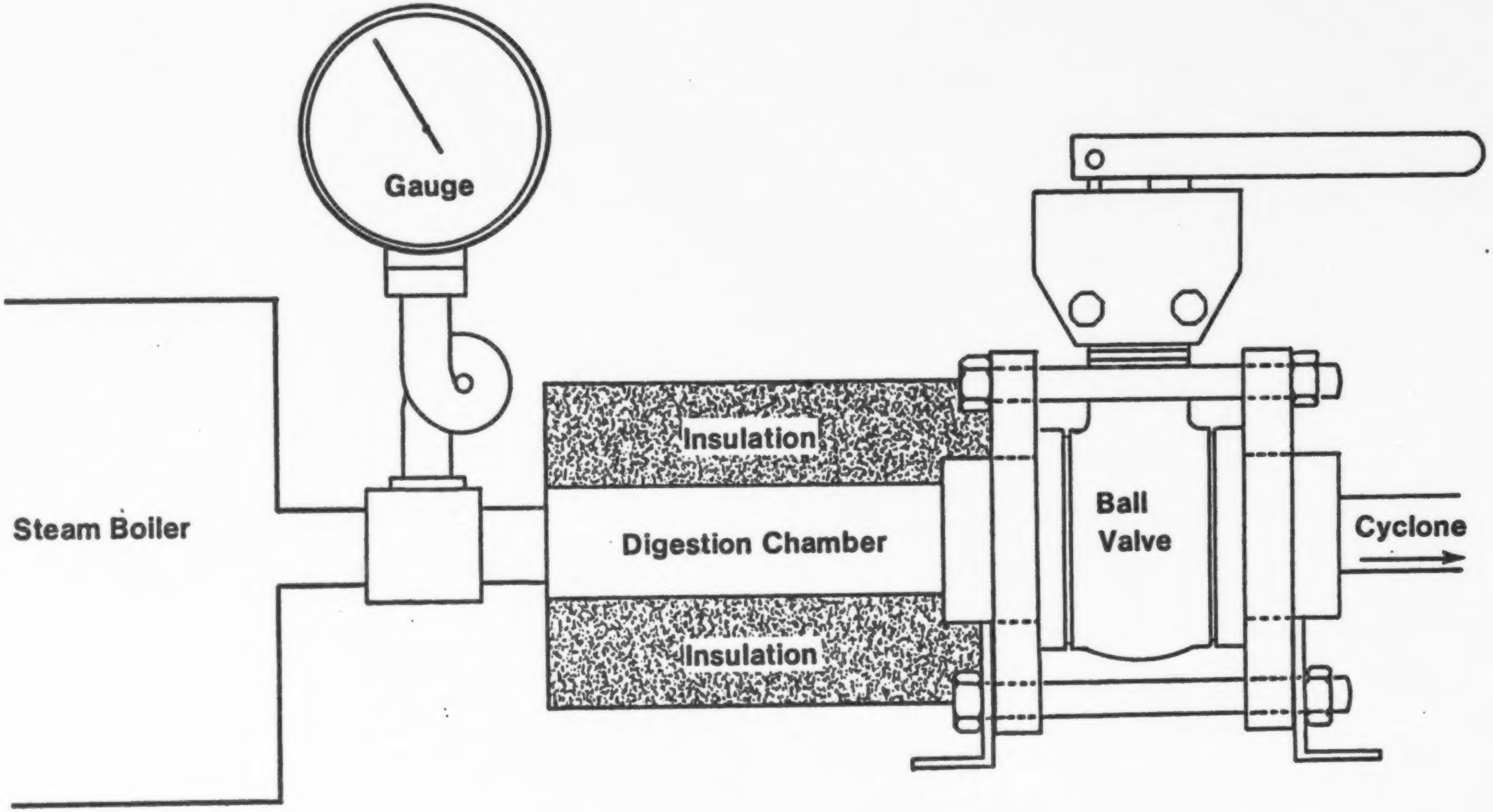

Gauge
Steam Boiler
Insulation
Digestion Chamber
Insulation
Ball
Valve
Cyclone

Figure 1. High Pressure gun for Steam Explosion (explosive decompression) of wood samples

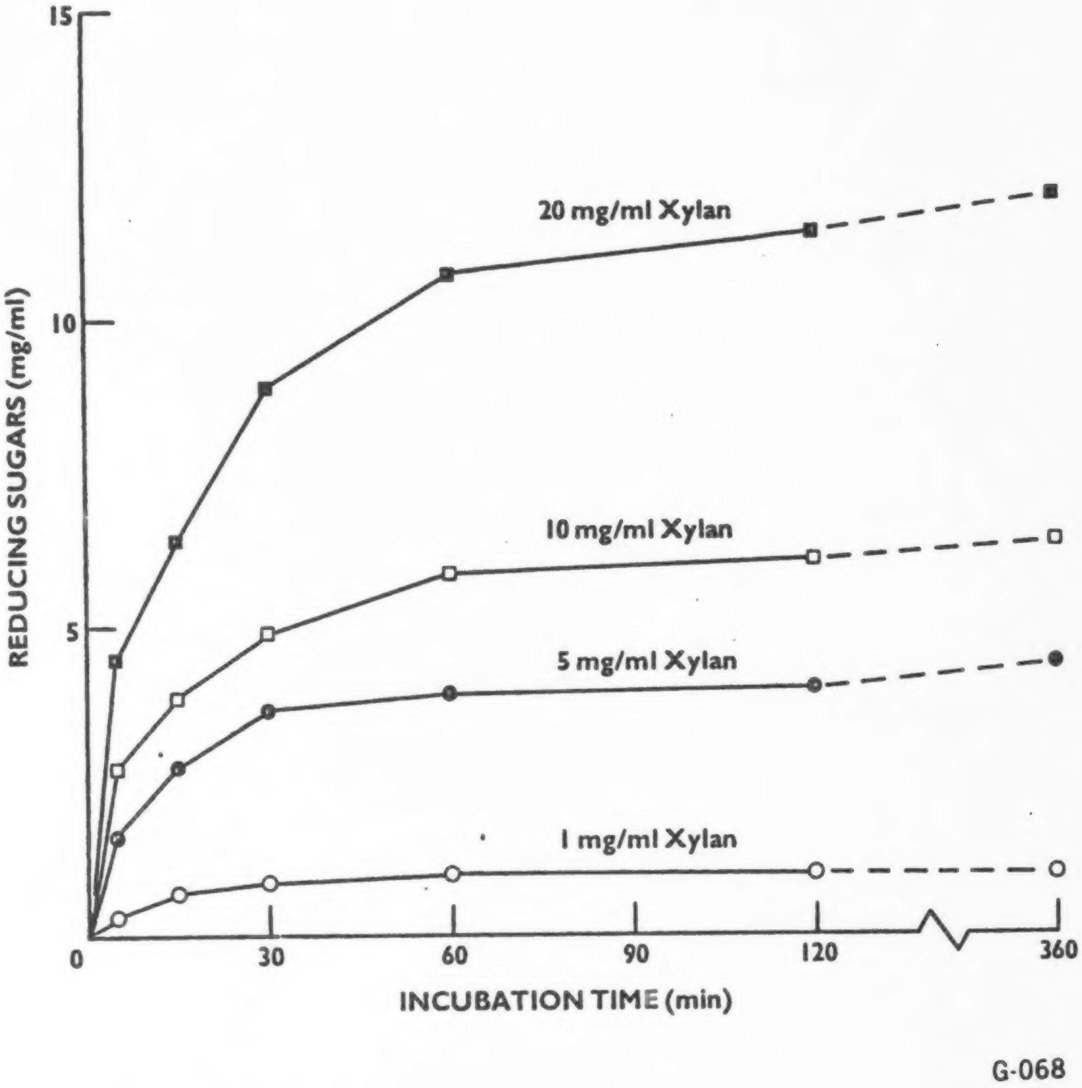


Figure 2. Hydrolysis of increasing concentrations of Xylan by Novo cellobiase

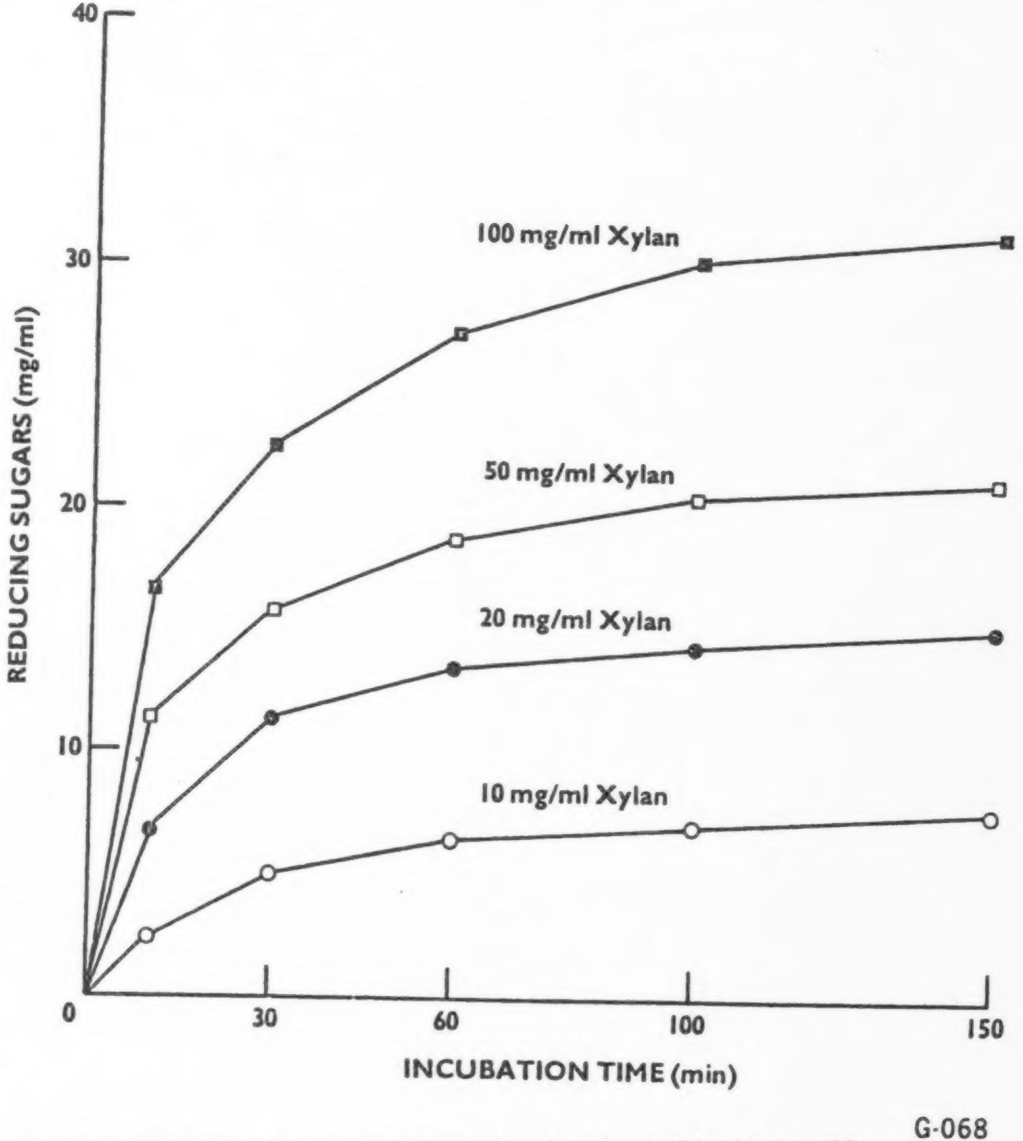
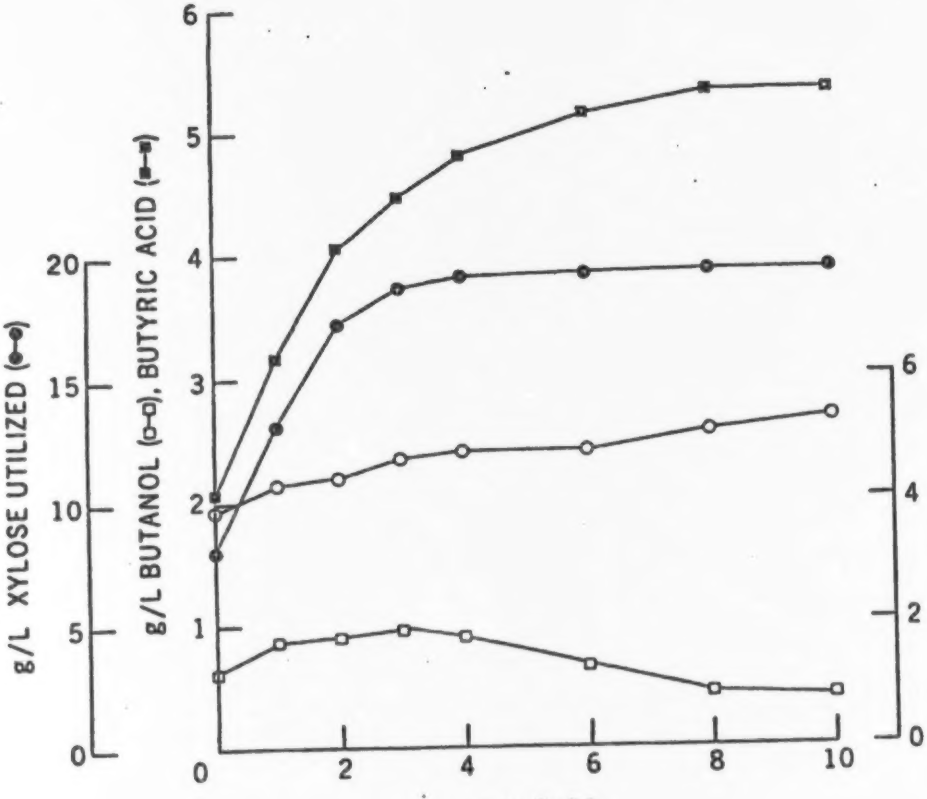


Figure 3. Hydrolysis of increasing concentrations of Xylan by *Trichoderma* E58 culture filtrate



G-073

Figure 4. Effect of $CaCO_3$ on xylose utilization and butanol production of C. acetobutylicum

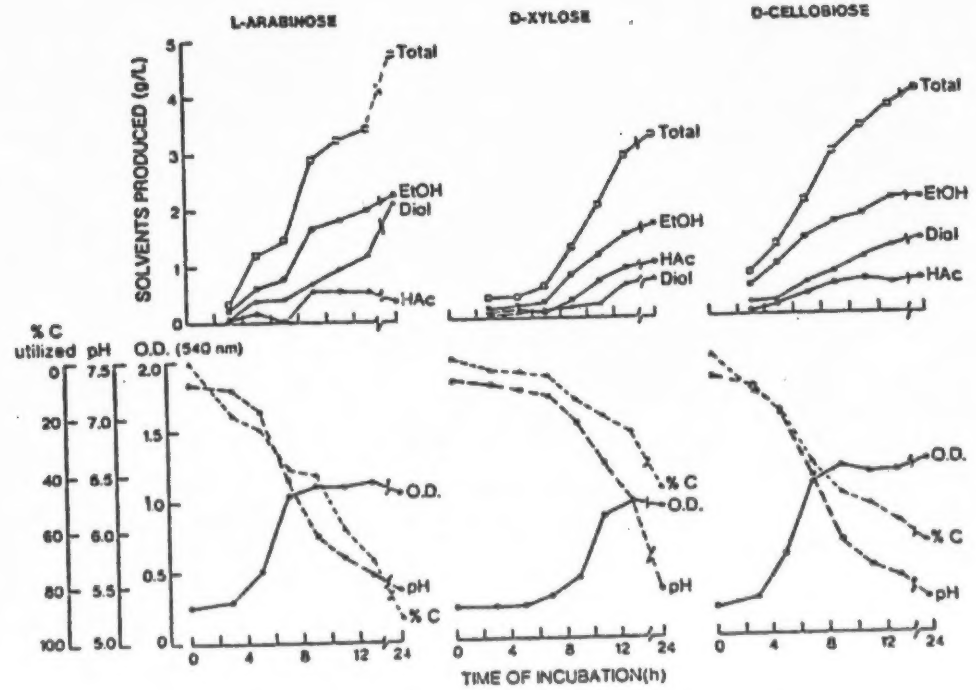


Figure 5. Growth and Solvent Production of *K. pneumoniae* on Pentoses and Cellobiose.

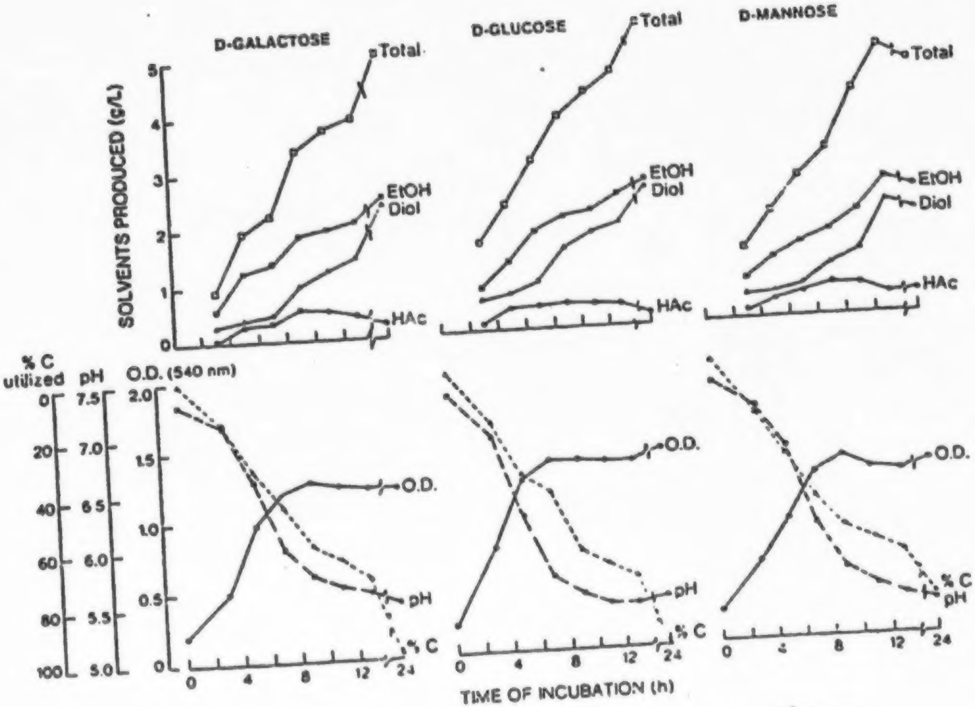


Figure 6. Growth and Solvent Production of *K. pneumoniae* on Hexose Sugars.

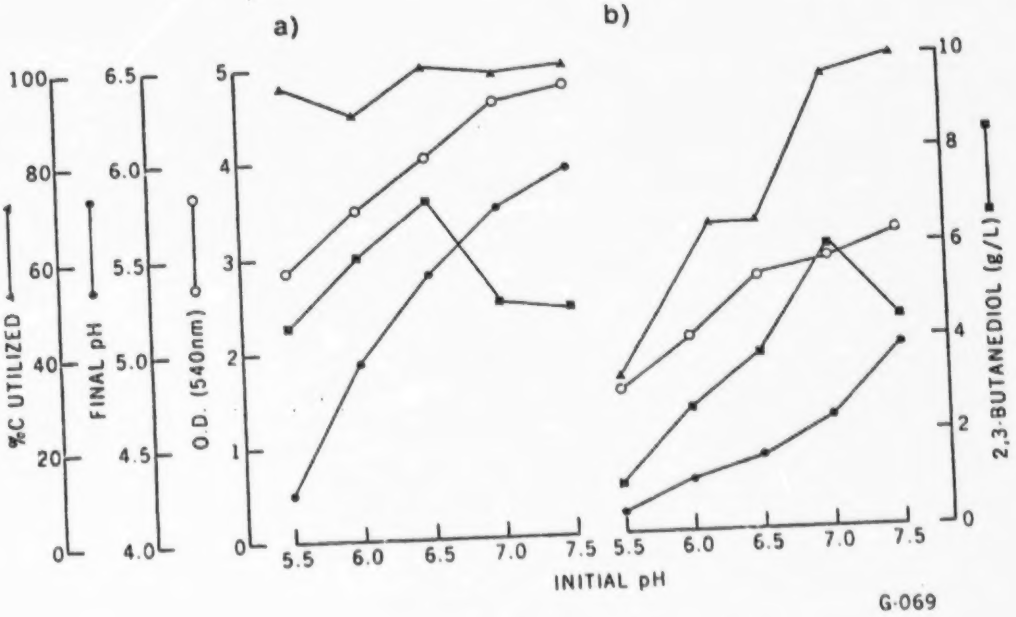


Figure 7. Effect of initial pH on growth and solvent production of *K. pneumoniae* grown on D-glucose at 30°C for 3 d under (a) finite air conditions, and (b) anaerobic conditions (N_2 atomosphere)

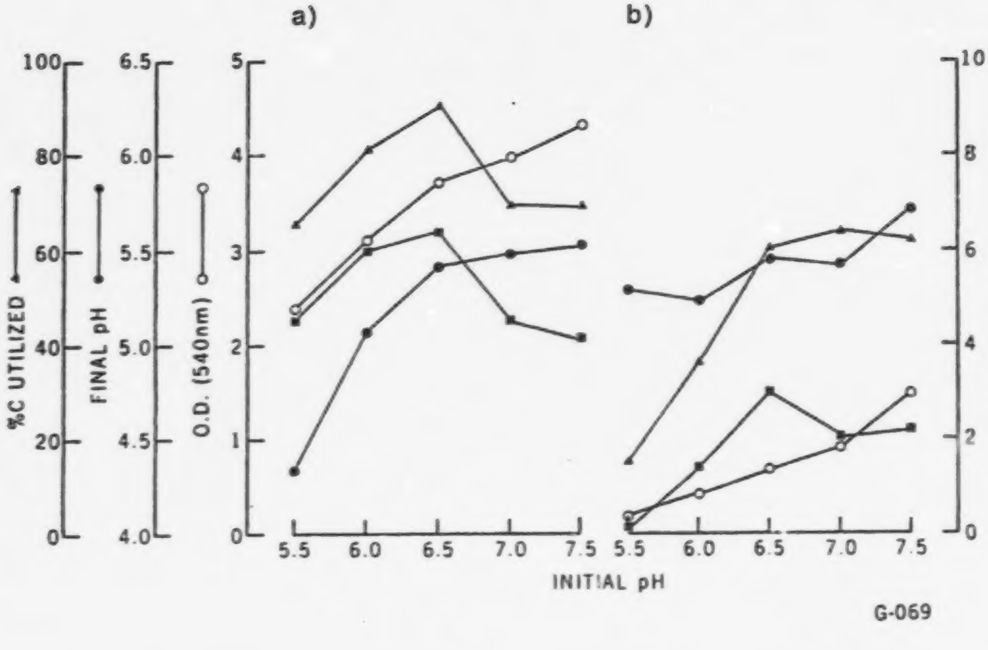
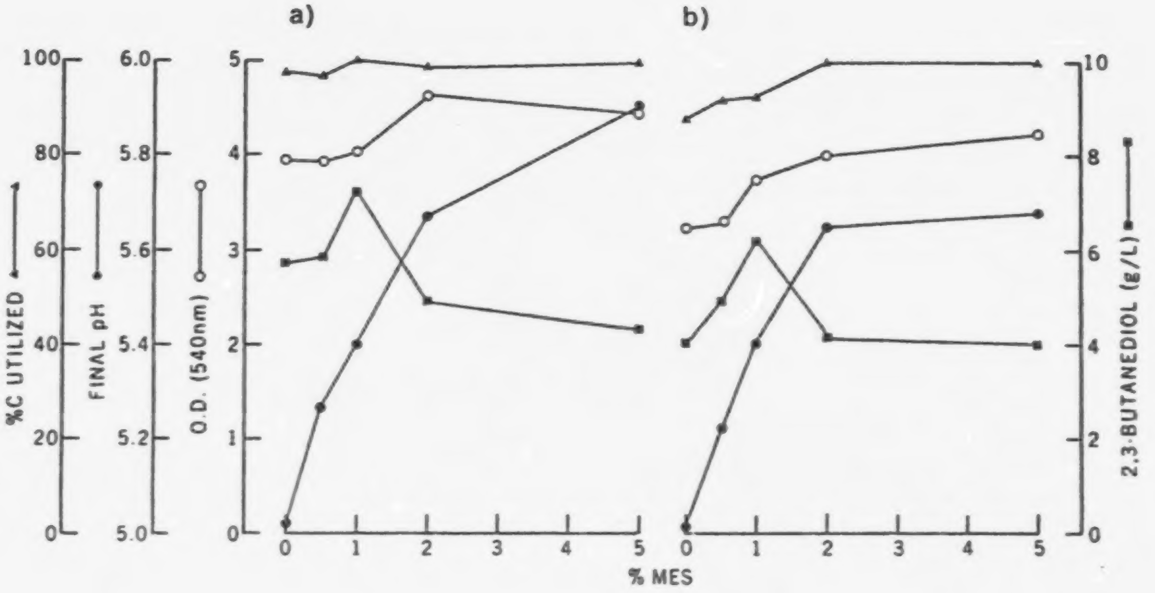


Figure 8. Effect of initial pH on growth and solvent production of *K. pneumoniae* grown on D-xylose at 30°C for 3 d under (a) finite air conditions, and (b) anaerobic conditions (N_2 atmosphere)



G-069

Figure 9. Effect of MES concentration on solvent production of K. pneumoniae grown under finite air conditions, within initial pH 6.5, at 30°C for 3 d, on (a) D-glucose and (b) D-xylose

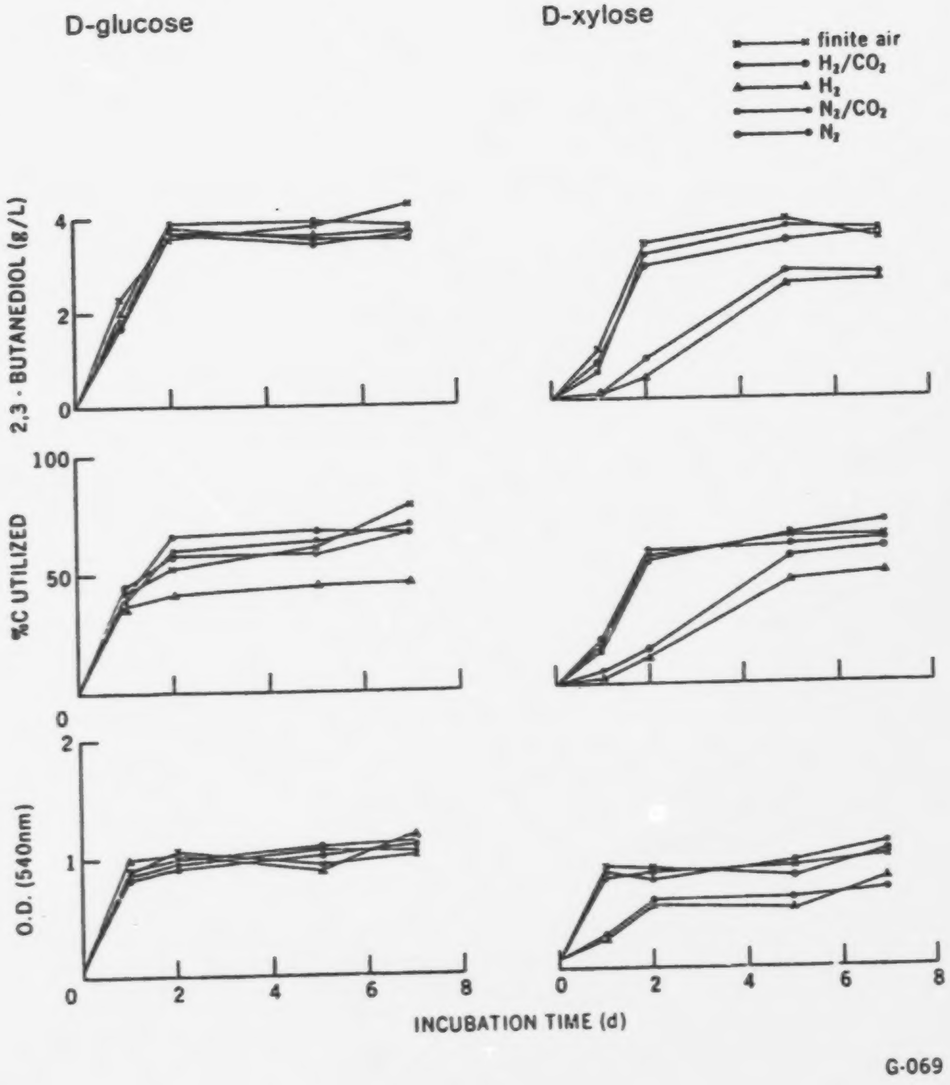


Figure 10. Effect of headspace gases on growth and 2,3 butanediol production of K. pneumoniae grown at 30°C, initial pH 6.5, under non-shaking conditions, with (a) D-glucose and (b) D-xylose as carbon source

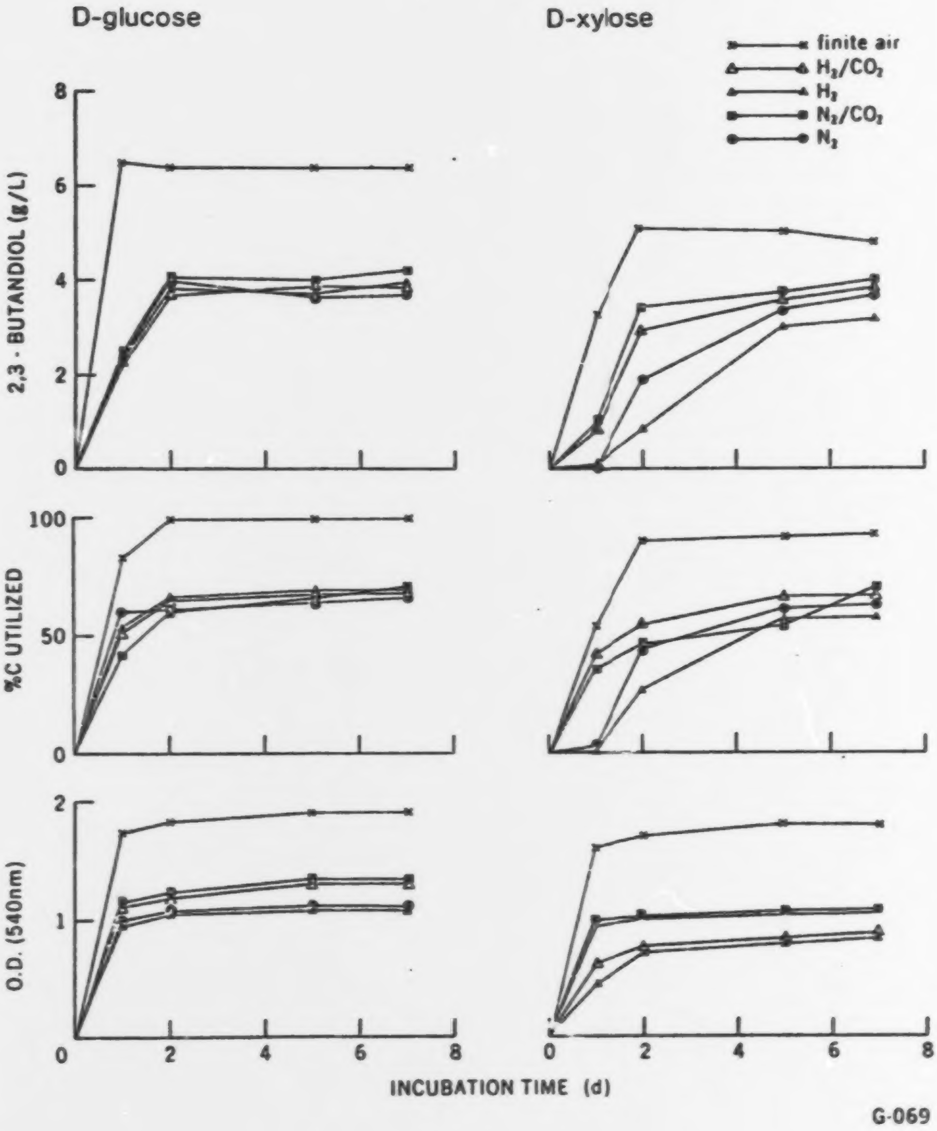


Figure 11. Effect of headspace gases on growth and 2,3 butanediol production of K. pneumoniae grown on 30°C, initial pH 6.5, under shaking conditions (120 rpm), with (a) D-glucose and D-xylose as carbon source

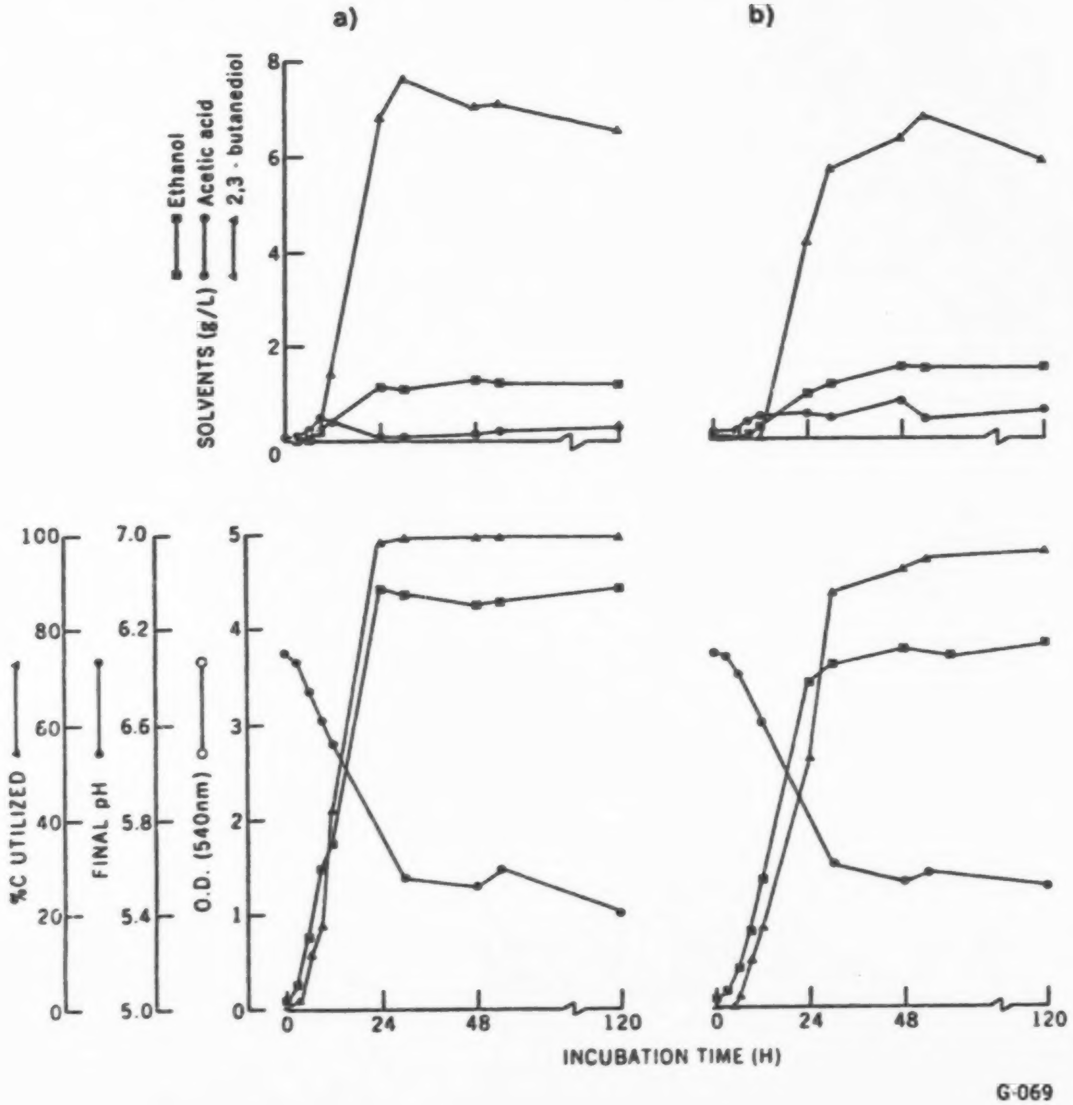


Figure 12. Time course of the growth and solvent production of *K. pneumoniae* grown under finite air conditions with initial pH 6.5 at $30^{\circ}C$ (120 rpm shaking) on (a) D-glucose and (b) D-xylose

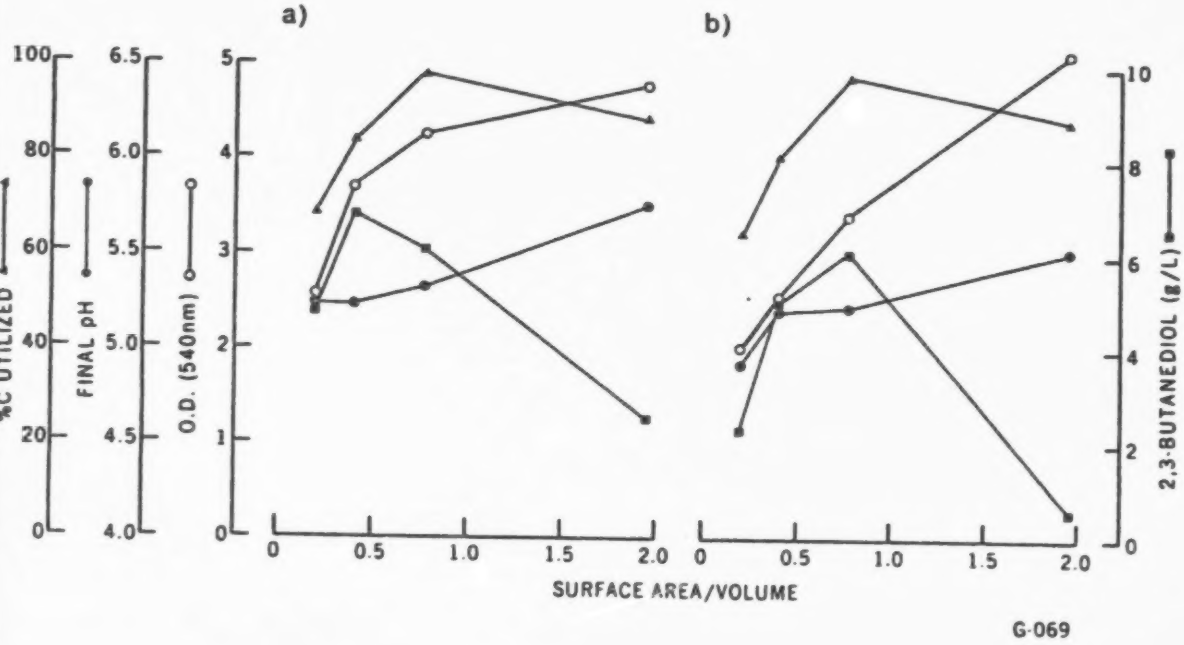


Figure 13. Effect of surface area to volume (A/V) ratio on growth and solvent production of *K. pneumoniae* grown at 30°C, initial pH 6.5, 120 rpm shaking, with (a) D-glucose and (b) D-xylose as substrates